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THE PHYSIOLOGICAL AND PATHOLOGICAL ASPECTS OF SILICA

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I. DISTRIBUTION OF SILICON. The element silicon occupies the same central position in inorganic nature that carbon occupies in organic nature. Next to oxygen it is the most abundant element. Although it is absent from the air, except as particles of dust, and is only a minor constituent of the hydrosphere, silicon is the element of chief importance in the lithosphere. It has been estimated that about 28 per cent of the solid crust of the earth is silicon. The figure 28 is also the atomic weight of silicon and if the amount of the element be expressed in terms of its oxide (silica-SiO_2), then it is seen that about 60 per cent of all rock is silica. The only important non-siliceous rocks are limestone and dolomite. Quartz and sandstone, gneiss and granite, slate and clay, are all compounds of silicon.

Silicon occurs as the free oxide silica, and in the form of silicates—metallic salts of its acids. Free silica is found in both the amorphous and crystalline state. Opal, tripoli and the deposits of diatomacious earth are composed of amorphous silica, containing a small percentage of water. Flint, chert, jasper and chalcedony are composed of amorphous silica associated with different proportions of crystalline silica. The chief types of crystalline silica are quartz, tridymite, and cristobalite. Quartz is the most common. It may be colourless (rock crystal): milky, owing to the presence of innumerable bubbles of air: smoky, due to carbonaceous material: or coloured, because of dissolved traces of metallic oxides. Tridymite is found less commonly in nature than is quartz. It is formed when quartz is heated a long time at about 1000°. It is formed during the manufacture of, and is found in silica bricks. So, likewise, is cristobalite, which is formed at a still higher temperature. The three kinds of crystalline silica exhibit different

crystal forms. Their specific gravities are: quartz, 2.67, tridymite 2.33, and cristobalite 2.34.

The silicates are the most important constituents of geological formations. Whole mountain ranges are composed of them. Chemically considered, silicates are the combined forms of silica with the oxides of metals, or the salts of bases with silicic acid. Silica is capable of existing in combination with water to form several silicic acids. In association with one molecule of water it exists as meta silicic acid (H_2SiO_3); with two molecules of water as ortho silicic acid (H_4SiO_4). By loss of water from two or more molecules of ortho silicic acid, condensed silicic acids may arise, thus disilicic acid ($H_6Si_2O_7$ from 2 H_4SiO_4) by the loss of 1 molecule of water, and trisilicic acid ($H_4Si_3O_8$ from 3 H_4SiO_4) by the loss of 4 molecules of water. The natural silicates found in rocks correspond to these silicic acids. Wollastonite ($CaSiO_3$) is a meta silicate; Willemite (Zn_2SiO_4) is an ortho silicate; serpentine ($Mg_3Si_2O_7$) a disilicate; and orthoclase ($KAlSi_3O_8$) a trisilicate.

Much of the natural silicate of the earth's crust has suffered a decomposition through weathering during geological time. The carbon dioxide dissolved in natural waters attacks those silicates which contain metals capable of combining with carbonic acid. Orthoclase, for instance, suffers disruption through its potassium being carried away in solution as potassium bicarbonate. Colloidal silica is at the same time liberated, and a residue of aluminium silicate (clay) is left.

II. The determination of silicon. Chemical methods for the estimation of silicon are gravimetric, titrimetric and colorimetric. All gravimetric procedures depend on the decomposition of the siliceous material to liberate silicic acid, which is subsequently dehydrated to the oxide, and isolated and weighed as silica.[1] The silica as isolated often contains small amounts of certain metallic salts which are difficult to eliminate. For this reason it is customary to treat with hydrofluoric acid which completely volatilizes the silica as silicon tetrafluoride (SiF_4). The loss in weight represents the true silica content of the sample.

[1] It is the custom to express the amount of the element in terms of its oxide-silica (SiO_2). This convention takes no account of the different forms in which silicon may exist in the materials under investigation. Only the total amount present is analyzed for and reported. It should, therefore, be borne in mind by those who are not familiar with analytical usage, that a figure for "percentage silica" (per cent SiO_2) gives no information as to how much free oxide of silicon is present. Except where used in some special sense and hence modified accordingly (e.g., "free silica"), the terms "silica" and "total silica" will be used in this article to signify total silicon (calculated as the oxide—SiO_2).

Most books on quantitative analysis contain adequate directions for the analysis of various siliceous substances. Hillebrand (67), Treadwell (161), and Scott (141) contain particularly full and valuable discussions of the many procedures which have been described.

The titrimetric determination of silicon (Tananaev and Babko, 156) depends on the reaction represented in the equation:

$$SiO_2 + 6NaF + 4HCl \rightarrow Na_2SiF_6 + 4\ NaCl + 2\ H_2O$$

The method is extremely rapid and easily executed (Koral and Kaluzhskaya 93). A good degree of accuracy can be obtained with fairly large amounts of silica, but in the experience of the authors it is not trustworthy as a micro-method.

Colorimetric methods for the determination of silica were introduced for the purpose of estimating the small amounts of silica in natural waters. The well-known Dienert and Wandenbulche (34) colorimetric procedure is based on the formation of a highly coloured yellow silico-molybdic acid. This reaction was first used for a colorimetric method by Jolles (71) in 1898, and has been variously modified according to the use to which it has been put by Thresh (158), Atkins (4), Thompson and Holton (157), who investigated waters and water supplies, and by King (83) who studied the silica content of animal tissues.

Reduction of the yellow silico-molybdic acid to give a blue complex has been made the basis of other colorimetric procedures. These have been principally used for examining animal tissues and body fluids, whose silica content is often very low, and which consequently require analysis by a very sensitive method. The blue colour developed from a given amount of silica is more intense than the corresponding yellow colour (cf. Roman 134, King and Stantial, 87; De Eds and Eddy, 32).

The quantitative determination of free silica in the presence of silicates presents many difficulties. The problem has been partly solved by utilizing the fact that the amorphous silica set free from many (but not all) silicates by boiling with hydrochloric acid is soluble in aqueous alkalies, whereas crystalline silica (quartz) is little attacked (Shaw, 142). Knopf (94) found that silicofluoric acid (H_2SiF_6) would dissolve the silicate components of rock dust leaving the quartz relatively unattacked. Silicoboric acid was preferred by Line and Aradine (105), who claimed a sharper separation than with silicofluoric. In the absence of an entirely satisfactory method of isolating mineral particles from tissue, these methods have had only a limited use in studying the nature of the dust deposits in lungs.

Spectrography has been applied to the study of the siliceous material in lungs by Policard (126). The x-ray diffraction methods have been applied by Clark and Reynolds (23) and Faber (38). By these means it is possible to determine the percentage of quartz in presence of other mineral substances. Hicks, McElroy and Warga (66) have used both methods for studying the dust deposits in human lung tissues.

III. Silicon in microörganisms. Diatomaceous earth consists of the siliceous skeletons of the microscopic plant life of the sea—the phytoplankton or diatoms. They have been called by Mann (107)"the grass of the sea," and form the most important food of aquatic animal life. In 1904 Richter (131) observed that artificial cultures of these marine organisms thrived in glass bottles but that very poor development took place in paraffined containers. From this it was concluded that silica is essential to their reproduction. Atkins (5) studied silica as a limiting factor in diatom growth in the sea, and Harvey (63), Coupin (28), King and Davidson (85) in artificial cultures. The subject has been reviewed by Harvey (61, 62), Calvert (19) and Cooper (27). Brussoff described a "bac. siliceus" capable of storing and depositing silica (17).

IV. Silicon in plants. Silicon appears to stand very much in relation to the skeletons of some plants as do calcium and phosphorus to that of animals. One family of sponges has a skeleton of almost pure silica. The ash of wheat straw may contain 40 per cent of SiO_2, and that of equisetum (common horse tail) has been shown to consist of up to 80 per cent SiO_2. The silica is usually deposited in the peripheral tissues of the transpiration organs and along the conducting vessels (Frey-Wyssling 43). Silica appears to have been largely superseded, during the evolutionary process, by the development of such stable organic substances as cellulose and lignin, which are capable of forming rigid structures (cf. 144). An extensive literature exists on the rôle of silica in plant growth and nutrition. This is reviewed by Nemec (119), Lemmermann (103) and Sreenivasan (146, 147).

V. Silicon in animal tissues. Silicon appears to be invariably present in animal matter. All tissues and fluids which have been examined contain at least traces, and in many instances the amounts found are of the order of those of other mineral elements which are recognized as being normal tissue constituents. The almost universal distribution of silica makes it probable that silicon will be at least a likely contaminant, if not an essential ingredient, of all protoplasm. All the marine forms examined have contained silica. The eggs of birds (35), milk (31) and the fetuses of mammals have been shown to

have small but appreciable quantities (88). In normal adult animal tissues it is present in somewhat larger amounts.

Schulz (1901) (136) made the first thorough investigation of the presence of silica in human material. He analyzed umbilical cord (137), thyroid glands (139), and pancreases (140), and found some small differences between normal and diseased tissues. Frauenberger (42) criticized some of his results as being too high, but with the analytical procedures then available for estimating the small amounts present in the tissues studied, some disagreement was to be expected. Gonnermann (1917) (47) regarded silicon as being of some importance in the metabolism of man and the higher animals. He greatly extended the analyses of tissues and investigated also the silica content of some important foodstuffs (48). This early work has been reviewed by Kuhn (1926) (99), in whose book will be found the results of a great number of investigations. A good summary of the position of silica in plant and animal physiology is also given by Lemay and Jaloustre (1925) (102). In this article are collected the results of many French investigations which are difficult to trace in the literature.

With the introduction of modern micro-methods of analysis the study of the distribution of silica was greatly facilitated. The use of the microbalance, the colorimeter and the spectrograph has rendered it no longer necessary to analyse large quantities of normal tissue in order to secure a determinable amount of SiO_2. The silica content of biological materials can now be determined easily and accurately on small amounts of tissue, which are preferable because of greater ease of manipulation in the analytical procedures. The results of analyses for a variety of material are collected in table 1. Where variable figures have been obtained by different investigators, the more recent results, or those which in the opinion of the authors, appear most reliable, have been included.

Inspection of the table indicates that the normal range of silica content for most tissues is from 4 mgm. to 40 mgm. per 100 grams dry fetal tissue, and from 10 to 200 mgm. for adult tissues. Lung is amongst the lowest in the fetal tissues, and muscle the highest. The reverse is the case in adult tissues. Normal human lungs and lymph nodes may contain large—in some cases enormous—amounts of silica (10). This is apparently derived from life-long inhalation of ordinary street and house dust, and tends to increase in concentration with age. In contrast is the constant low concentration of silica in other organs and in muscle, which does not appear to vary appreciably during life. Kahane and Antoine (73) have called this the "silice de constitution"

TABLE 1

Silica content of normal tissues

FETAL TISSUES	SiO_2 DRY TISSUE	ADULT TISSUES (HUMAN)	SiO_2 DRY TISSUE
	mgm. per 100 grams		*mgm. per 100 grams*
Whole mouse[7]	10	Heart: myocardium[1]	11–29
Blood (human)[8]	13	Heart: endocardium[1]	14–28
Brain (human)[7]	22	Ileum[1]	47
Heart (human)[7]	20	Kidney[1]	11–27
Kidney (human)[7]	13	Liver[7,1]	11–17
Kidney (calf)[7]	8–10	Lung[7,8,9,4] (infancy to old age)	10–200
Liver (human)[7]	4–6	Lung[1] (infancy to old age)	14–2000
Liver (calf)[7]	4–10	Lymph glands[1] (peribronchial)	27–5000
Lung (human)[7]	8–10	Mammary gland[1]	12
Lung (calf)[7]	4–10	Milk[6,10]	71
Lung (human)[8]	34	Muscle: pectoral[1]	15–45
Muscle (calf)[7]	24–37	Muscle: psoas[1]	23
Spleen (calf)[7]	11	Nerve: peripheral[1]	11–40
ADULT TISSUES (HUMAN)		Nerve: spinal cord[1]	13–87
Adrenal[1]	14–80	Ovary[1]	10–21
Appendix[1]	68	Oesophagus[1]	8–26
Aorta thoracic[1]	14–34	Pancreas[1,5]	14–31
Artery iliac[1]	14–50	Parathyroid[1]	22–23
Bladder[1]	7–21	Pituitary[1]	27–67
Bone[7,1,3]	12–30	Placenta[8]	29
Brain: dura mater[1]	11–37	Prostate[1]	11
Brain: cortex[1]	12–36	Rectum[1]	15–74
Brain: Cerebellum[1]	16	Skin: scalp[1]	35–270
Brain: pons[1]	13–43	Skin: abdomen[1]	40–130
Brain: pineal[1]	70–90	Skin: sole of foot[1]	11–59
Cecum[1]	20–27	Spleen[1]	15–41
Cartilage: coastal[1]	18–35	Stomach[1]	12–42
Cervix[1]	11	Teeth[2]	6
Diaphragm[1]	11–50	Tendon, Achilles[1]	12–16
Duodenum[1]	10–80	Thymus[1]	81
Gall bladder[1]	31	Thyroid[1]	21–55
		Testes[1]	8–20
		Umbilical cord[1]	33
		Uterus[1]	7–12

[1] Belt et al. (10).
[2] Bowes and Murray (14).
[3] Chapman (22).
[4] Fowweather (41).
[5] Kahle (74).
[6] Kettmann (76).
[7] King et al. (88).
[8] McNally (116).
[9] Sladden (145).
[10] Strohecker et al. (151).

and contrast it with the "silice d'interposition" which comes from inhaled siliceous particles. They have isolated birefringent particles from the blood and from other organs than the lungs, showing that small amounts of particulate matter may find their way from the pulmonary apparatus into the general circulation. This exogenous silica appears as crystalline fragments, leaving a residue after treatment with hydrofluoric acid. In contrast the endogenous silica is amorphous and dissolves completely in hydrofluoric acid. It is likewise soluble in aqueous alkali, whereas Badham and Taylor (7) have pointed out that mineral particles are not so attacked.

The nature of the "silice de constitution" has been a matter of conjecture. Most authors consider it a simple deposit of hydrated silica. The claim of Drechsel (36) to have isolated a cholesterol silicate has not been confirmed (21). Johlin (72 a) deduced evidence for the presence of a silico-organic compound by electro-dialysis of gelatin, tendon, blood and urine. A jelly-like material containing both silicic acid and carbohydrate appeared at the cathode. Colloidal silica, on the other hand, migrates to the anode. Only very small amounts of the substance were obtained and it was not possible to determine its exact nature.

Many claims have been made for the presence of abnormal amounts of silica in diseased tissues, e.g., Kühn (99), Robin (133), Murata and Kageyama (117), Sztankay (154, 155). Anderson and Dible (1) have recently (1938) suggested an etiological relationship between silica and malignancy. A group of cases of pulmonary carcinoma was examined in which the organs contained an excess of silica and exhibited histological evidence of silicotic fibrosis. The elasticity of connective tissue has been held to be associated with its silica content, which, it is claimed, decreases with age (95), and with the concurrent loss of elasticity. This association furnished a rationale for the extensive silicic acid therapy which was practised for many years, particularly in central Europe. Thus A. Kühn (98) treated arteriosclerosis, bronchial asthma and increased blood pressure with injections of silica-containing solutions. Broman (1923, 16) questioned the influence of silica on the elasticity of connective tissue. She failed to find any relation between the SiO_2 content and the tensile strength of tendons of rabbits, or to find any evidence that silica administration strengthened the healing tendon.

The elaborate claims of many authors (e.g., 97, 75, 95) for a beneficial treatment of tuberculosis of the lung with silicic acid preparations are, in the opinion of the writers, devoid of any adequate proof. The whole

question of silica therapy has been critically reviewed by Maver and Wells (108).

VI. Silicon in blood. In 1857 Witting (168) recorded 0.5 per cent of SiO_2 in the ash of blood. Gonnermann (47) reported 2 per cent in whole blood, 1 per cent in the serum, 3 per cent in the cells, and 16 per cent in the ash of fibrin. Numerous other results are given by Kuhn (99) from the older literature. Kraut's (1931) average figure of 1.8 per cent SiO_2 in the ash of a whole blood for 32 normal persons is in close agreement (96), but Lematte and Kahane's (101) analysis of dried fibrin and blood showed a much smaller content of silica than that given by previous authors.

The custom of recording the silica content as a percentage of the ash makes it difficult to compare many of the recorded results (particularly in the German literature) with those expressed as milligrams per 100 cc. of blood. Only where departures from the normal are claimed can comparisons be made. King et al. (88) found by a colorimetric method (confirmed by gravimetric) that the average silica content of human blood was a little less than 1 mgm. SiO_2 per 100 cc. The blood of several animals yielded very similar figures. Pribyl's results for the blood serum of cattle, sheep, goats and pigs were of the same order (128).

Kraut (96), using a different method of analysis (wet-ashing of 5 cc. of blood, followed by HF treatment of the "sulphate ash" with measurement of the loss in weight due to volatilization—presumably of silica), found a very much higher concentration in the blood. Normal men and women varied between 10 and 30 mgm. SiO_2 per 100 cc., with an average value of 16. Miners with and without silicosis, and tuberculous patients, had considerably more (Böhme and Kraut 13). Reckoned on an ash basis Kraut's figures agree fairly well with the older German results, but they are greatly (at least 10 times) in excess of values determined elsewhere (86, 116, 134). This large discrepancy is probably to be explained on the basis of the technique of analysis used for the silica determinations.

King and Dolan (86) were unable to confirm the finding of an increased blood silica in cases of tuberculosis or silicosis, or in miners exposed to a high concentration of quartz dust in the air. This was in keeping with the findings for animals (89) which were injected with silica and in which no appreciable increase of blood silica could be demonstrated, despite large increases in the amounts in the urine. It was considered that a person exposed to the inhalation of silica dust would, if he dissolved any of it in his lungs, promptly excrete it in his urine.

The claim of Gürsching and Kraut (56) to have found a relationship of the blood silicic acid to the parathyroid glands and the serum calcium could not be confirmed by the authors.

Changes in the silica content of the blood following administration of silicic acid esters have been repeatedly demonstrated (89, 44). It is possible that when present as an organic compound silicic acid can remain at high concentration in the blood when it is incapable of remaining there as inorganic silicate.

VII. Absorption and excretion of silicon. Whether or not silicic acid administration can lead to an increase of the blood silica, there can be no question that absorption of silicic acid takes place, followed by excretion in the urine. Schulz (138) and Salkowski (135) found that silica introduced into the organism with the food is excreted in large measure in the urine, and Gonnermann (49) that the drinking of water rich in silicic acid leads to increased urinary excretion of silica.

The silica content of flours and grains was investigated by Berg (11). In general it may be said that the brans of grains contain more silica than the bolted flours; and hay and straw contain considerable amounts. For this reason herbivorous animals are ingesting much larger quantities of silica than are meat-eating animals. Much of this silica is eliminated in the feces, but a considerable proportion must be absorbed, as is shown by the constant high excretion in the urine. King et al. (88) found that herbivorous animals excrete ten to thirty times as much silica in their urine as do carnivorous animals. The amount of the excretion could be varied at will merely by changing the diet. When the feed of rabbits was changed from oats and hay to white bread and tomato juice, the urinary silica promptly fell to low levels, to be restored again to its previous high level with the restoration of the high silica diet. With human subjects Riessner and Kindt (130) found a similar result when sodium silicate was incorporated in the bread. They claimed (and likewise Kockmann and Maier 92) that a retention of part of the administered silica in the organism took place.

The administration of pure silicic acid, or of neutralized sodium silicate into the stomach leads to an increase in the urinary silica (see fig. 1). Whatever silica enters the blood stream by way of the intestine appears to pass rapidly into the urine, there building up to a high concentration without any significant increase of the level in the blood. By continuous intravenous injection of soluble silica in isotonic sodium chloride (89) it was possible to increase enormously the concentration of silica excreted in the urine (up to 150 times the original), without any very marked increase in the blood. In unpublished experiments on the

absorption and excretion of silicic acid in human subjects King and Brock found large, although variable, amounts of silicic acid to pass from the gut into the urine without demonstrably increasing the level in the blood. These findings seem to indicate that the body possesses a very efficient mechanism for ridding the blood of its dissolved silica. There was also some evidence that parenterally administered silicic acid may be excreted in part by the gut as well as by the kidneys.

VIII. Solubility of silica. The modern concept of solubility as a figure designating the composition of a liquid phase which is in a state of dynamic equilibrium with a solid phase, constitutes an intelligible

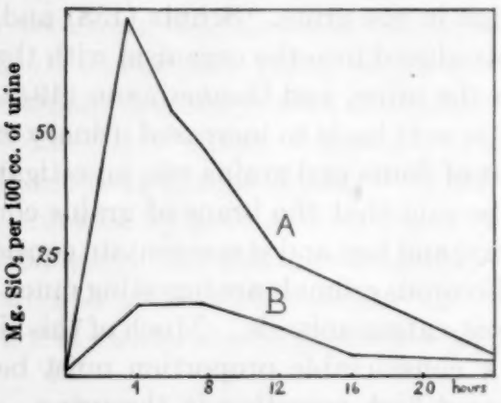

Fig. 1. Effect of administration in the dog's stomach (A) of silicic acid, and (B) of powdered quartz, on the excretion of silica in the urine (after King and Dolan, 86).

picture for systems consisting of gelatinous silicic acid suspended in liquids. But the applicability of this concept to the system represented by a large crystal of quartz in distilled water is not clear. That some silica may have passed from the crystal into the liquid is possible, but the presence of silica in the water is a matter of presumption since there is no means of demonstrating its presence there. It can only be said that the properties of the liquid are approached by those of a silicic acid solution as it becomes more and more diluted by the addition of larger and larger amounts of water. In the limit the presence of silicic acid is certain, but its amount is immeasurable; and its presence in any small portion of the whole is a matter of chance. Some such reasoning is probably the basis of the belief that silica is "insoluble" in water, except in so far as that belief is modified by the conception of all substances having some solubility, be it ever so small.

The "insolubility" of silica, however, does not stand the test of experiment. If the quartz be broken into coarse fragments and suspended in water, the liquid may acquire properties which make it appear that some silica has passed into solution, and if it be reduced to a fine powder then the presence of measurable amounts of silica in the liquid phase becomes determinable. But the measurement of the dissolved silica presents grave technical difficulties because of the difficulty of separating liquid phase from solid. When the solid phase consists of extremely fine particles—less than 1 micron in diameter, then much of it is found to be in a state of more or less permanent suspension. The removal of these fine particles from the liquid defies most technical means which are available. Simple filtration is useless. For this reason the early results on solubility of quartz (e.g. Strückmann 150a) are not a true measure of the silica which is present in a state of molecular dispersion. Lenher and Merrill (1917) recognized this fact in their solubility studies, but had no means of overcoming it (104). Their reported figures are considerably higher than those given by later workers. Only through high-speed centrifugation—at 10,000 to 20,000 r.p.m. (84), or through ultrafiltration—forcing the liquid through a membrane of fine porosity under many atmospheres' pressure (160), can the suspended fine particles be completely removed.

The utilization of a chemical reaction into which only the molecularly dispersed silicic acid will enter furnishes a further means of obtaining a measure of the dissolved silica. Such a reaction is represented in the silico-molybdic acid method which is the basis of most of the colorimetric procedures for silica. Silica solubility figures determined in this way are open to the objection that the reagents may have a solvent effect on particles of undissolved silica, but in point of fact the results so obtained agree very closely with those determined on ultra-centrifugates and ultrafiltrates.

A peculiarity of silica solutions is that they do not appear to become "saturated" except in the presence of a great excess of very finely divided solid. The "solubility" varies, furthermore, with the state of division of the solid (Lucas and Dolan, 106; Briscoe et al., 15). The material used in the solubility experiments (90) illustrated in figure 2 was very finely powdered quartz, prepared from rock crystal which analyzed almost 100 per cent SiO_2. Despite the fact that this specimen of quartz appeared to be a very pure one, it did not obey the simple phase rule criterion of purity which states that the addition of further solid to a saturated solution causes no increase in the amount of dissolved mate-

rial. An equilibrium seems to be reached within a few days between the liquid and any small amount of solid which may be shaken with it. But this apparently saturated solution will dissolve more silica, up to a point, as more quartz is added and shaken with it. It is possible that an additional degree of freedom may exist in the system, viz., there may be another component in the form of hydrated silica existing on the surface of the particles, and it may be this which is the actual material passing into solution. But even this explanation is not satisfactory since it might be expected that as hydrated silica was dissolved from

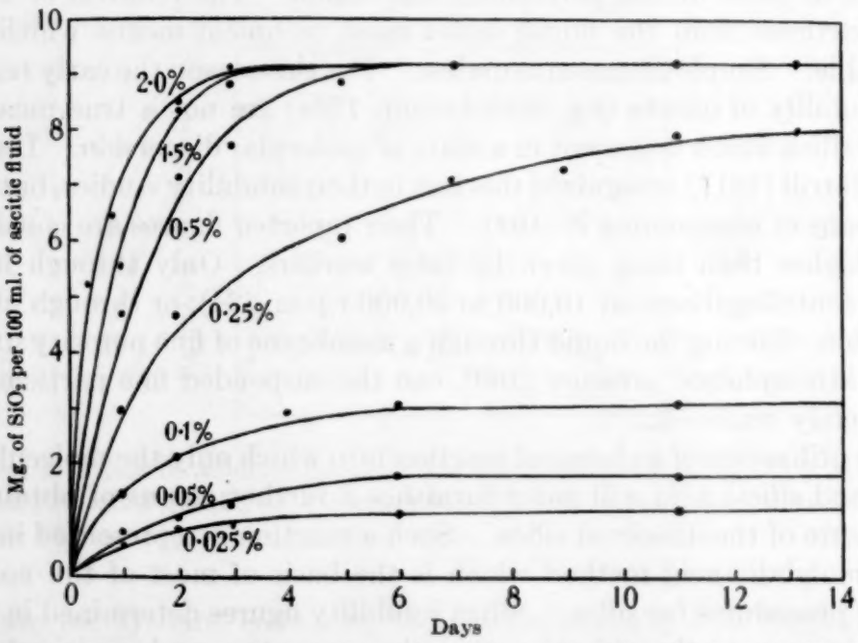

Fig. 2. Effect of concentration of solid on the dissolution of silica. Percentage figures represent grams of rock crystal dust suspended in 100 ml. of ascitic fluid (after King and McGeorge, 90).

the surface of any particle, another layer of it would form. Furthermore, it is found (Lucas and Dolan, 106) that the re-suspension in fresh solvent of the solid material, which has been separated from a "saturated" solution, results in the dissolution of an amount of silica which is equal to that in the first solution. And yet neither solution is saturated in the ordinary sense, since more silica will be dissolved if more solid be added. The total surface of solid exposed probably determines the rate, if not the final amount of silica dissolved, and the whole phenomenon might better be considered as a leaching of material from the surface of particles, than as a simple dissolution.

The vexed question of whether dissolved silicic acid exists in a state of colloidal or molecular dispersion (either in artificial solutions, natural waters, or biological fluids) (6, 166) has been at least partially resolved by combined high-speed centrifugation and ultra-filtration experiments (90). In every instance where an appreciable amount of silica was shown by analysis to be present in the liquid, there was a lower result obtained in the ultrafiltrate than in the centrifugate. The difference was in general greater the larger the amount of dissolved silica. The membranes used were capable of filtering off such colloids as plasma globulin and albumin, and it is probable that the silicic acid removed was present as a colloid of somewhat similar dimensions. It would seem likely, with a feebly ionized substance like silicic acid which is known to polymerise easily that reversible transitions from the molecularly dispersed state to the colloidal are usually taking place in its solutions.

In table 2 are collected the results of several investigators for the amounts of silicic acid dissolved from the different forms of silica. In water and biological fluids most of the reported figures for amorphous silica vary between 5 and 14 mgm. per 100 cc. of solution and for quartz between 4 and 10 mgm. The value for silica solubility recorded by Titus (160) is very much lower, but this was obtained with very much less solid phase than was used by most other workers. The naturally occurring silicates do not appear, in general, to be as soluble as the free forms of silica in neutral or alkaline liquids. In an acid medium some silicates are dissolved to a considerable extent.

Several substances act as depressors of silica solubility. These fall into two classes—certain metals or their oxides, and substances like coal which adsorb silicic acid from solution and hence appear to depress solubility. The metals and metallic oxides (Al, Fe, Ca, Mg, etc.) appear to precipitate silicic acid as fast as it goes into solution as the insoluble silicate of the metal. Aluminium and iron are particularly efficient depressors of solubility (Denny et al., 33). The effect is best seen on the inclusion of a small amount of the finely divided metal with powdered quartz in water. Minute traces only of dissolved silica can be demonstrated in the liquid, even after many days shaking.

IX. Silica in lungs. Since the original demonstrations of Peacock (1860, 121), and of Greenhow (1865, 55 a) of "sand" in human lungs, the study of the accumulations of siliceous material in the tissues has been the subject of numerous investigations. Only a few of them can be mentioned here (for a résumé of the literature see McNally, 115).

TABLE 2

Dissolution of silica in various fluids

FORM OF SILICA	*grams solid per 100 cc.*	DISS. SiO_2 *mgm. per 100 cc.*	SOLVENT	EXPOSURE	TEMPERATURE *°C.*	AUTHOR AND METHOD
Amorphous						
Gelatinous	2*	9.0	Serum	1 week	37	Ultra-centrif. (90)
Gelatinous	2	9.1	Ascitic fluid	72 hours	37	Ultra-centrif. (40)
Dry	2.5	10	Water	10 days	37	Colorimetric (3)
Desiccated	2	7.0	Ascitic fluid	8 days	37	Ultra-centrif. (90)
Fresh	2	9.0	Ascitic fluid	8 days	37	Ultra-centrif. (90)
Fresh	2.5	14	Pleural fluid	10 days	37	Colorimetric (3)
Quartz						
Quartz	1	3.3	Water	8 days	37	Colorimetric (106)
	4	3.8	Water	8 weeks	20	Colorimetric (15)
	1	5.2	Water	3 hours	100	Colorimetric (15)
	0.01	0.05	Water	2 months	37	Ultra-filtr. (160)
	1	4.3	Water	10 days	37	Colorimetric (3)
		5	Water			Colorimetric (118)
	3	19	1 per cent $NaHCO_3$	48 hours	37	Colorimetric (161)
	3	18	0.1 N NaOH	24 hours	37	Colorimetric (161)
	1	2	0.1 N HCl	24 hours	37	Colorimetric (161)
"Bull" quartz	2	6.4	Ascitic fluid	8 days	37	Ultra-centrif. (90)
Rock crystal	2	9.1	Ascitic fluid	8 days	37	Ultra-centrif. (90)
	1	4.5	Pleural fluid	10 days	37	Colorimetric (3)
Flint	1	24	Water	3 hours	100	Colorimetric (15)
	1	43	1 per cent $NaHCO_3$	48 hours	37	Colorimetric (161)
	1	18	0.1 N NaOH	27 hours	37	
	0.3	2.7	0.1 N HCl	24 hours	37	Colorimetric (161)
	2	6.5	Ascitic fluid	8 days	37	Ultra-centrif. (90)
Granite	3	5	1 per cent $NaHCO_3$	48 hours	37	Colorimetric (161)
Felspar	1	3.4	Water	3 hours	100	Colorimetric (15)
Orthoclase	1	1.3	Water	7 days	37	Colorimetric (106)
	1	0.8	0.9 per cent NaCl	7 days	37	Colorimetric (106)
	2	0.8	Ascitic fluid	8 days	37	Ultra-centrif. (90)
Sericite	1	2.2	Water	3 hours	100	Colorimetric (15)
	0.01	0.05	Water	2 months	37	Ultra-filtr. (160)
	0.01	1	Serum	2 months	37	Ultra-filtr. (160)
	0.1	0.8	1 per cent $NaHCO_3$	8 days	37	Colorimetric (84)
	2	0.8	Ascitic fluid	8 days	37	Ultra-centrif. (90)
	0.1	0.4	1 per cent $NaHCO_3$	4 hours	100	Colorimetric (84)
Mica	1	0.7	Water	14 days	37	Colorimetric (84)
	1	0.45	1 per cent $NaHCO_3$	14 days	37	Colorimetric (84)
	2	1.8	Ascitic fluid	16 days	37	Colorimetric (90)

* The figures in the first column give the amount of solid phase taken per 100 cc. of solvent.

TABLE 2—*Concluded*

FORM OF SILICA		DISS. SiO_2	SOLVENT	EXPOSURE	TEMPERATURE	AUTHOR AND METHOD
	grams solid per 100 cc.	*mgm. per 100 cc.*			°C.	
Asbestos	1	25	Water	3 hours	100	Colorimetric (15)
	2	1.6	Ascitic fluid	8 days	37	Ultra-centrif. (90)
Fuller's earth	2	1.4	Ascitic fluid	8 days	37	Ultra-centrif. (90)
Kaolin	2	0.4	Ascitic fluid	8 days	37	Ultra-centrif. (90)
		0.3	Ascitic fluid	16 days	37	Colorimetric (84)
Talc	2	0.8	Ascitic fluid	8 days	37	Ultra-centrif. (90)

Kussmaul (1867, 99a) considered that the silica of lung tissue was entirely exogenous and that it only appeared after several months of life. While this view can no longer be held (89, 116), it is true that there is a gradual accumulation of siliceous material in the pulmonary tissues from youth to old age and particularly in persons habitually exposed to the inhalation of siliceous dust. Extensive analyses of lungs of persons with and without dust exposure have been made by McCrae (111), McNally (115), Antoine (2). Workers in dusty trades—miners, stonecutters, pottery workers, grinders, sand-blasters, etc., are apt to suffer an extraordinary accumulation of mineral particles in their lungs; and the lungs of dwellers in the sand-storm regions of the Sahara desert have been shown by Policard and Marrion (125) to have notable deposits of siliceous particles. The amount is usually expressed as a percentage of the total silicon (as SiO_2), contained in the ash or dry tissue. Collins and Dible (24) have pointed out that a figure giving the total silica contained in the lung is a better expression of its mineral content than is a percentage. Antoine (1) found a large degree of variation in the siliceous particle content of different parts of the same lung. The amount is greater in the region of the lower lobe than the upper and is greatest in the region of the hilum. It seems to be concentrated in the root glands whose content may reach 14 times that of the parenchyma.

The average content of silica in the lungs of normal adult persons ranges between about 40 and 200 mgm. of SiO_2 per 100 grams of dry

tissue, i.e., 0.04 to 0.20 per cent. Hackmann (59) considered that in cases of occupational exposure to dust, injuries to the tissues are usually observed when the silica content exceeds 1 per cent. It was held by Sladden (145) that extensive fibrosis is usually found when the silica content is 1 to 1.6 per cent, while an amount in excess of this is said to be almost invariably accompanied by severe fibrosis. These conclusions have been criticised by Belt, Irwin and King (10) (cf. also Fowweather, 41), on the ground that the lungs of non-silicotic miners showed equally as much analyzable silica as the lungs of others who died with massive silicotic fibrosis. In the case of peribronchial lymph glands an accumulation of silica was found to take place with age, which might reach the very high figure of nearly 5 per cent SiO_2 in the dry tissue without any real fibrosis having developed.

The mineralogical nature of the siliceous material in the lungs has concerned many workers. McCrae (111) and Watkins-Pitchford and Moir (165), made a detailed analysis of the ash of silicotic lungs. As might be expected they found not only free silica, but mineral silicates. Jones (72) showed that a high proportion of the mineral particles may consist of sericite, an aluminium potassium silicate of the mica family. Sundius et al. (152, 153) have made detailed analyses of the dust isolated from lungs of workers in dusty trades. In their second paper references are made to many of the investigations of the mineral matter from asbestosis lungs. The subject of mineral dust particles is thoroughly dealt with by Drinker and Hatch in their book, *Industrial Dust* (1936) (37).

It has been frequently postulated that part of the silica tended to become hydrated in the lung to form gelatinous silicic acid—the "occult silica" of Watkins-Pitchford and Moir, 1916, and Belt, 1929 (cf. Heffernan, 65; Irwin, 70). Tideswell (159) and Badham and Taylor (7) attempted to estimate this hydrated silica by chemical means. Its relation to the production of silicotic fibrosis has been a matter for repeated speculation (cf. Faber, 39, who claims x-ray spectrum evidence for the presence of colloidal silica in lung tissue).

X. The dissolution of mineral dusts in vivo. The pathogenicity of silica in relation to silicosis is thought by many people to depend on its solubility. There has accumulated a body of evidence (dealt with in a later section) which seems to imply that dissolved silica has a poisonous effect on the tissues. If the harmful effect of silica in the cells is related to its dissolution, then it might be expected that the silica particles in a silicotic lung would dissolve to some extent in the fluid bathing

them, or at least suffer some leaching of soluble silica from their surfaces. Such a dissolution of silica in the lungs should result in the passing of silicic acid into the blood, followed by its excretion in the urine. The evidence in this connection is conflicting. The inhalation of amorphous silica may lead to a prompt increase in the silicic acid of the urine (91), but inhalation of powdered crystalline silica has led to only minor increases. Only by giving massive doses in intratracheal insufflation experiments with animals (86) was it possible to demonstrate a urinary excretion of extra silicic acid. King and Dolan (86) found the average silica excretion to be higher in a group of gold miners than in a non-mining population, and Bloomfield and Sayers (12) claimed to have shown a relation between the silica dust exposures of miners and the amount of silica in their urines. But it seems likely, on the basis of data at present available, that only a small proportion of the total silica in the urine may come from the lungs. Dietary silica so readily causes variations in the urinary silica, that the food and drink of silicotics would have to be very rigidly controlled before any significant changes due to the dissolution of silica in the lungs could be looked for. The daily differences in urinary silica levels may be quite as great in an ordinary urban population (46) as among miners, or indeed as between individual miners and persons not exposed to the inhalation of excessive dust. An attempt was made to study the 24-hourly excretion of silica in carefully controlled groups of silicotic and nonsilicotic tuberculous patients in a sanitarium (86). No significant differences were observed. But this failure does not preclude the possibility that some of the very finest of the silica particles breathed by a worker in a dusty atmosphere may undergo a fairly prompt dissolution when they come in contact with the fluids of the lung. Larger particles may be dissolved much more slowly—too slowly, in fact, to show any evidence of dissolution other than a slow change in their physical characteristics as revealed by microscopic study.

The increased urinary silica values observed in persons inhaling large amounts of quartz dust may be related to a dissolution of particles in the intestinal tract as well as in the lung. There can be no doubt that large amounts of dust are coughed up (Burke, 18) and swallowed, and it has been shown that the administration of powdered quartz into the stomach of a dog leads to increased excretion of silica (fig. 1). The absorption of silica in the gut from mineral dusts has been studied by Whitehouse (167), who administered to himself samples of powdered rock. These were suspended in distilled water and swallowed. Only

in the case of Scotch whinstone (a rock of mixed silicates) and of very finely divided flint dust was he able to show a significant rise in his urinary output of silicic acid. Working with much larger samples of stone dust King and McGeorge (91) showed that several other siliceous minerals were dissolved to an appreciable extent in the gut and excreted in the urine of cats. Most of the free forms of silica showed this increase and some of the silicates. Those of the latter which appeared to dissolve were silicates which are easily decomposable by acid. They were probably attacked by the hydrochloric acid of the stomach. The resulting amorphous silica would easily be soluble in the alkaline duodenal juice.

Intraperitoneal injection of suspensions of the dusts led to the interesting contrast that whereas the free silicas seemed to be dissolved and excreted, the silicates were attacked to only a minor extent. Those silicates which had led to increased urinary output of silica after oral administration failed to give any comparable result when inoculated into the abdominal cavity. But here the dusts were subjected only to a simple leaching process by the mildly alkaline body fluids, without any preliminary exposure to an acid treatment such as they suffered in passing through the stomach. In all cases where dissolution appeared to have taken place, the excretion of extra silica was only transitory, and only a small proportion of the total silica injected was recovered in the urine.

Although the amounts of mineral dust administered in these experiments (91) were so great as to represent an abnormal exposure, they can probably be taken to indicate that some dissolution of siliceous minerals normally takes place in the body.

XI. EVIDENCE THAT SILICA[2] DUST IS HARMFUL TO MAN is based largely upon epidemiological investigations of miner's phthisis and other allied forms of occupational tuberculosis. Such epidemiological surveys as have been made in this field by Haldane (60), Collis (25), Hoffman (68), Lanza (100), Middleton (112), have strongly suggested an etiological relationship between silica and dust phthisis. On the strength of this evidence the term silicosis has largely supplanted the earlier usage of the term "miner's phthisis."

[2] It is unavoidable that we should have to use the terms silica and siliceous dust more or less interchangeably in a rather loose sense, much in the same way that chemists use the term total silica (see footnote page 330) without trying to indicate a specific compound of silicon (see Belt, Irwin and King 10).

If any one man more than another stands out as the father of the modern conception of silicosis, it is the late Professor J. S. Haldane, whose able investigations into dust hazards are summarised in the Second Report of the Royal Commission on Metalliferous Mines and Quarries, 1914, (60). To this obscure publication the better known and widely quoted Milroy Lectures of Collis, 1915, (25) appear to owe their inspiration. Much of the epidemiological spade work with respect to silicosis which is commonly attributed nowadays to others may be traced to Haldane. The gist of Haldane's thesis was "If in any class a high death-rate from pulmonary tuberculosis is found occurring at a later period of life than is usual for pulmonary tuberculosis and if this high death-rate is associated with a high death-rate from other respiratory diseases, then this class is exposed to the inhalation of injurious dust." The one factor which seemed to be common to all the injurious dusts was silica; therefore the Report concludes "The cause of miners' phthisis is the inhalation of dust of crystalline silica, upon which is superimposed tubercular infection."

Thus, the industrial hazards of siliceous dust first became apparent by virtue of an excessive incidence of pulmonary tuberculosis amongst the affected peoples. To this day, tuberculosis is perhaps still the best index of a harmful dust (Kettle, 1934) (77).

Silicosis may be defined as an industrial or occupational disease of the lungs, sporadic in incidence, insidious in onset, of great chronicity, occurring amongst workers who are exposed for years to the inhalation of siliceous dust. It is characterised essentially by extensive nodular fibrosis and by a marked tendency on the part of its victims to die eventually of pulmonary tuberculosis. "I believe the disease is the same all over the world," said Haldane (60), and most other authorities support this view.

The tissue changes produced in the lung may be and often are of a complex nature, but the essential lesion is a distinctive type of nodular fibrosis, the unit of which is commonly referred to as the silicotic nodule.

XII. THE MORPHOLOGY OF THE SILICOTIC NODULE. The silicotic nodule is a small, firm, rounded, tubercle-like structure varying roughly from 1 to 5 mm. in diameter and distributed unevenly through the lung parenchyma. Under the microscope it appears as a more or less spherical focus of dense, inert, acellular, avascular, collagenous connective tissue. Its component strands of collagen are relatively coarse and tend to be hyalinized. They have a concentric arrangement and often appear to be interwoven like basketwork. They are commonly described as

forming a whorled structure. Some of the American authors have coined an apt simile in comparing the nodule to a miniature ball of twine.

While its morphology is distinctive and, in general, more or less constant, the silicotic nodule has a good many variable features: it is more often conglomerate than single; it may have a necrotic centre or be partly calcified; its outline may be sharply defined or it may be fringed by an irregular mantle of cellular connective tissue; even its dust content lacks a specific constancy (see below).

By the very nature of its variations and by the relative imprecision of histopathological terms it is not possible to phrase an exact definition of the silicotic nodule. It is not surprising, therefore, to find that even amongst the ablest pathologists, there is not strict uniformity of opinion as to the morphology of the lesion. It will be especially important to bear this in mind when we come, presently, to discuss the experimental reproduction of silicosis.

The unparalleled experience of silicosis in the Witwatersrand Gold Mines of South Africa has given rise to some of the most authoritative studies we possess on the pathology of the disease. To such experts in this field as Watkins-Pitchford (163), Mavrogardato (109), Irvine (69) Strachan and Simson (148), we may turn for the most representative illustrations of the silicotic nodule (see plates 1, 2 and 3).

XIII. The dust content of the silicotic nodule. Characteristically, there is some degree of black pigmentation due to particles of anthracotic dust in the interstices of the nodule (see Watkins-Pitchford, 163). This black, carbonaceous dust is probably quite fortuitous in respect of the silicotic nodule, but it represents a convenient indicator of the less obvious, colourless, siliceous dust with which it is usually intimately mixed (see Belt, Irwin and King, 10).

Compared to other parts of the silicotic lung, the nodule itself usually appears to contain relatively little silica; with the polarising microscope there appear to be fewer and smaller siliceous particles within the nodule than in the cellular connective tissue around it (Belt, 8). This appearance is deceptive for the nodule usually contains a characteristic deposit of altered (occult) silica which can only be unmasked by some such process as microincineration (Watkins-Pitchford and Moir, 165).

Microincineration is, at least theoretically, the method of choice for demonstrating silica deposits in tissue sections (see Irwin, 70). By this means Policard and Martin (124) have made a quantitative histochemical study of the ash of silicotic nodules. They calculate that the

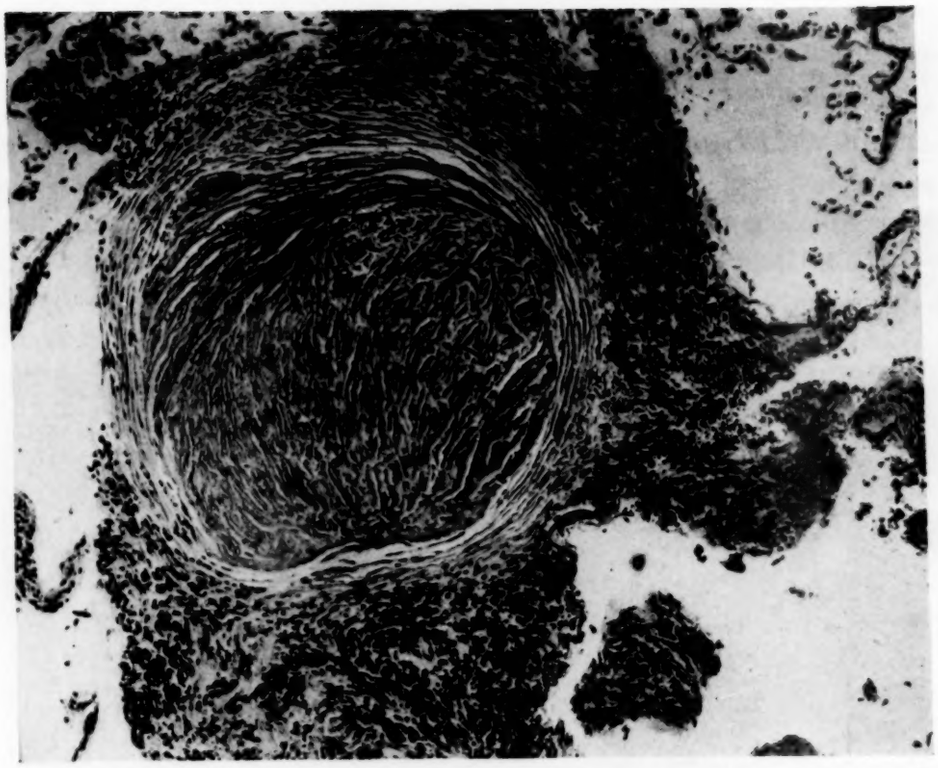

Plate 1. Photomicrograph (magnification about 50). After Simson and Strachan (150). Described as "Fully formed, single, small 'non-infective' silicotic nodule."

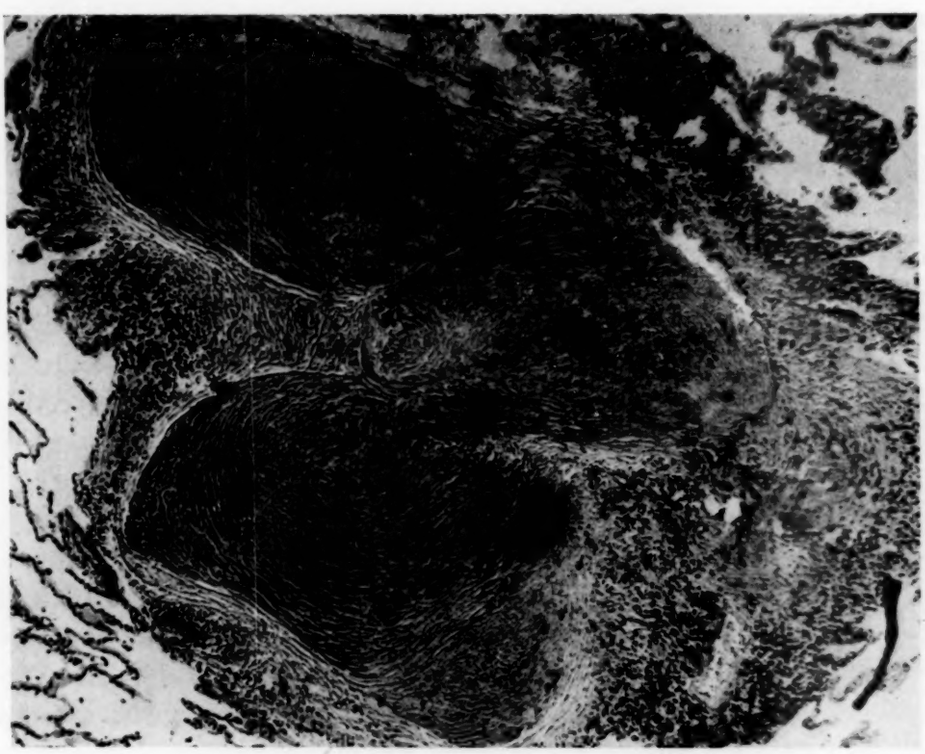

Plate 2. Photomicrograph (magnification about 35). After Simson and Strachan (150). Described as "Composite 'non-infective' dilicotic nodule."

siliceous residue represents about 5 per cent of the dry weight of the average nodule. This siliceous ash is a white amorphous powder, the like of which is peculiar to the silicotic nodule; it probably derives from inhaled particles which have undergone some chemical transformation in the nodule. Policard (132) believes it has undergone actual chemical

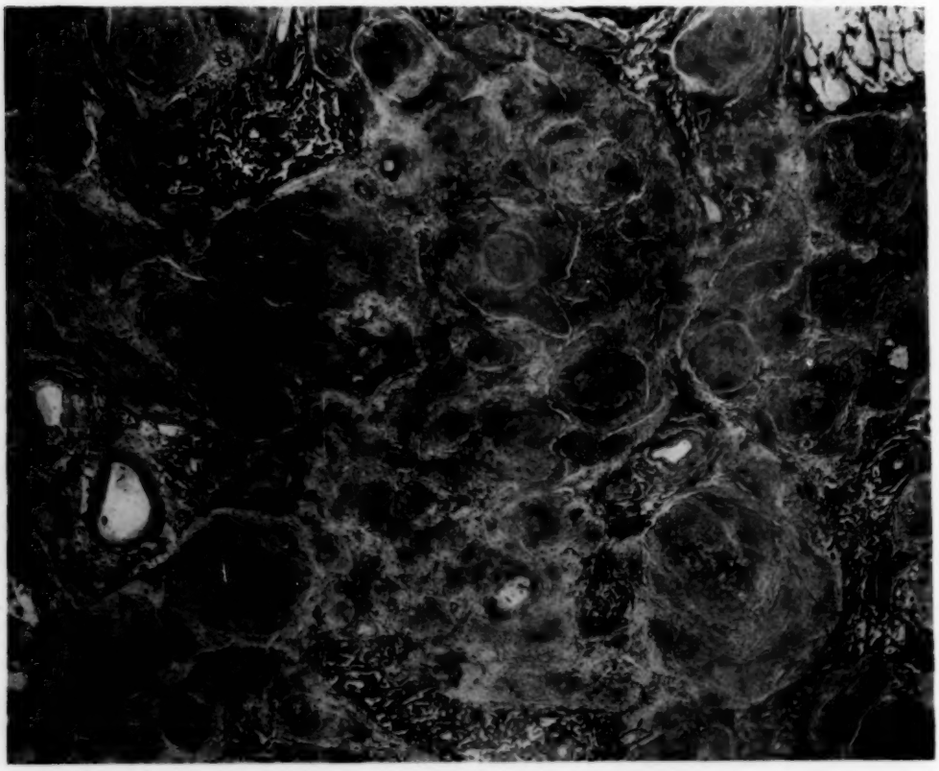

Plate 3. Photomicrograph (magnification about 10). After Simson and Strachan (150). Described as "Silicotic massive fibrosis of 'non-infective' type, composed of numerous contiguous, composite and single silicotic nodules."

combination with the collagen and refers to it as "du type des cendres de constitution, résultant des matières minérales faisant partie de la constitution même du tissu incinéré."

While the quality of the silica deposit appears to be more or less constant from one silicotic nodule to another, the quantity varies considerably (Policard, 124) and probably bears no constant relationship to the size of the nodule. Moreover the dust deposit does not permeate the whole nodule uniformly. Thus there is apparently more fibrosis in the silicotic nodule than could be explained as a simple foreign body reaction. In other words, the silicotic nodule represents a redundant

fibrous reaction, manifesting a bulkier proliferation of fibrous tissue than would be essential for segregating the amount of dust involved.

The size of the harmful particles. By digesting the tissue (or tissue ash) with strong acid and recovering the mineral matter McCrae (111), Watkins-Pitchford and Moir (165), Giese (45), Policard (123) and Jones (72) have studied the size and shape of siliceous particles in the pneumoconiotic lung. There is general agreement that the majority of such particles are of the same order of size as common micro-organisms (Mavrogardato, 109). The average dimension is about 1.μ or less. Particles larger than 12.μ have rarely been observed in the pneumoconiotic lung except in the case of asbestos which, quite unlike other dusts, enters the lung in the form of long fibres, sometimes 100 to 200μ in length, Cooke (26), Gardner (52 a). The small size of the silica particles probably has a good deal to do with their pathogenicity. Gardner and Cummings (54) have shown that particles of quartz under 3μ are much more productive of connective tissue proliferation (liver cirrhosis) than particles of larger size. The smaller the particles, the more available they become for chemical change, and the greater is their relative solubility (Lucas and Dolan, 106; King and McGeorge, 90). The small size of the particles found in the lung is of such an order as to facilitate optimum availability, a factor of great importance since the pathogenicity of silica may be related to its solubility (see p. 338). Middleton (113) has pointed out that the most harmful dusts in industry appear to be those which contain a high proportion of very small particles. From the point of view of an industrial hygienist he believes the greatest damage is caused by particles under 1μ in size.

XIV. THE PATHOGENESIS OF THE SILICOTIC NODULE is related in some way to the presence in the lung of excessive quantities of siliceous dust, but how the lesion develops is still largely a matter of conjecture. Kettle (78) said "Now, in most forms of chronic fibrosis we are able to give some reasonable account of the development and progress of the lesion. By a histological analysis of, say, the various stages of the contracted granular kidney, we can describe pretty well how the condition is produced, but we have a good deal to learn about the development of the pulmonary lesions in silicosis. . . . Experimental investigations might have been expected to help in the elucidation of the process, but for the most part they have not done much to clear the air, and this is not for want of time or trouble."

Surprising as it may seem, it is nonetheless true that great difficulty

has been encountered in tracing the various stages of development of the silicotic nodule in human lungs. The full-blown nodule is well recognised but the early stages are but sketchily defined. This may be due to several factors: 1, not many cases of early silicosis come to autopsy; 2, the nodules in any given case tend to mature synchronously so that there is often little variation from one lesion to another; and 3, the end-stage picture is often so complicated by tuberculous infection that it is difficult to distinguish tuberculous from dust lesions.

The spherical morphology of the nodule indicates a focal origin at a small central point around which the lesion is built up concentrically; but it is difficult to postulate any such focal starting points in the topography of the dust-ridden lung for the dust is usually widely disseminated throughout the pulmonary lymphatic apparatus. In short, the distribution of the dust is more diffuse than focal and therefore provides no obvious clue to the origin of the focal, nodular lesion.

The distribution of the nodules might be expected to offer some indication as to what particular parts of the lung are most vulnerable to silica and perhaps indeed it does. In fact it is generally conceded that the lesions develop in the pulmonary lymphatic apparatus, first in the glands, then along the linear lymph channels which parallel the blood vessels, bronchi, inter-lobular septa and pleura. But one has only to consult Miller's (114) recent monograph on the anatomy of the lung to realize with what a complicated structure one is dealing in the pulmonary lymphatics and how ill-defined they usually are in ordinary histological preparations of the lung. When one reflects with what difficulty the anatomists themselves have delineated the finer structural details of the normal lung, it is not surprising that pathologists working with the distorted anatomy of the silicotic lung have been puzzled to know in what particular part of the lung lobule the silicotic nodule takes origin. In the absence of better than ordinary histological methods of identifying the smaller lymph channels and lymph nodes of the lung parenchyma, the so-called lymphatic distribution of silicotic nodules is little more than a hypothetical conception.

On the basis of their wide acquaintance with the morbid anatomy of the silicotic lung, Simson and Strachan (149) suggest a constant anatomical relationship between the sites of 1, early aggregation of dust cells, and 2, subsequent nodule development, and that these sites correspond to hypothetical outposts of the pulmonary lymphatic apparatus, namely, small lymph nodes situated at the bifurcation of each terminal bronchiole at the entrance of each lobule. Gardner (53) favours this

view. If such an hypothesis be tenable then there may be said to exist some anatomical basis for the nodular morphology of the silicotic lesion.

These parenchymal outposts of the lymphatic system are visualised as miniature lymph follicles which act as dust traps; the dust-laden phagocytes, migrating from the air sacs towards the hilum or the pleura, collect in these traps, where, presumably, after prolonged residence, small aggregations of siliceous dust provoke their characteristic nodular reaction.

As to the more specific pathogenesis of the silicotic nodule there are two current theories, somewhat at variance, which must occupy our attention. The first of these, which may be called for the sake of convenience, the "silica specificity theory" ascribes the nodule to a specific effect of siliceous dust per se, while the second, which may be styled the "infective theory," invokes a dual etiology, namely, siliceous dust and tubercle bacilli.

1. *The silica specificity theory* regards siliceous dust as the sole etiological agent of the silicotic nodule. Adherents of this theory, who are probably in the majority, have sought to define the modus operandi of silica on a physical or chemical basis. Thus, until comparatively recently it was held generally that silica owed its harmful properties to its physical characters; the hard, sharp particles were supposed to damage the pulmonary tissue, and the whole process was regarded as a kind of microscopic trauma which after prolonged action in minute foci produced redundant fibrosis. Haldane is said to have abandoned this mechanical concept only when Kettle (79) showed that flint particles were rendered innocuous by a thin coating of iron oxide which did not alter their sharpness. Equally convincing is the fact that carborundum dust with particles just as hard and sharp as silica, apparently produces no comparable lesion (Gardner, 50). Thus the theory of mechanical irritation has been replaced by the view that silica becomes soluble in the tissues and exerts a chemical rather than a physical effect. It must not be presumed, however, that the physical presence of silica particles is altogether a matter of indifference to the tissues, for the mere presence of foreign particles always tends to set up a process of phagocytosis and segregation by fixed tissue proliferation, as is well-known in the case of common pigmentations like tattooing, anthracosis and hemochromatosis. In such pigmentations the reaction is of the simplest foreign-body type, and the same reaction necessarily must take part to some degree in the tissue response to siliceous dust, but in the latter instance, as we have seen, the reaction tends to outreach these simple

proportions and become redundant. For the redundant qualities of the silicotic nodule, therefore, some process other than a foreign-body reaction must be called to account.

Simson and Strachan (149) who, in common with most of the other present day investigators in Africa, regard the silicotic nodule as a dust effect pure and simple, trace various histological stages of nodule development in the human lung.

Their statement of what is presumed to take place may fall short of enabling a precise visualisation of the process but it is widely credited as an adequate explanation, namely, that the silicotic nodule springs, tumour-fashion, from a nidus of dust cells by an indolent proliferation of fibroblasts whose growth is in some way initiated and stimulated by a small aggregation of siliceous dust particles. It is agreed generally that silica must undergo dissolution to produce this effect.

Gardner (53), whose unrivalled experience of dusting experiments places him amongst the foremost authorities on the subject, believes that silica, during prolonged residence in the tissue, gives rise to a poisonous solution which causes cell damage, necrosis, lymph stasis and chronic inflammation, and that these changes are obligatory antecedents of the full-blown nodule.

There is experimental evidence for believing that certain forms of soluble silica have a mildly toxic effect upon endothelial and connective tissue cells. Thus Gye and Purdy (58), were able to produce liver cirrhosis in rabbits by the parenteral administration of repeated small doses of colloidal silica, and Gardner and Cummings (54) duplicated this feat with finely ground quartz. Subcutaneous inoculations of silica particles in mice produce what Gye and Kettle (57) called "almost a specific lesion" characterised by acute inflammation and necrosis. Similar results are reported by Fallon and Banting (40). Mavrogardato (109) found evidence for believing that silica had a "mummifying" effect upon the phagocytes which ingested it, and Policard shares this view (122).

On the basis of these experiments and others too numerous to mention it is judged probable that the toxicity of silica may be held accountable for the development of silicosis in man. This hypothesis becomes the more tenable since, as we have indicated in the earlier part of this paper, it is now accepted that silica is appreciably soluble in body fluids (see p. 338). The toxicity theory postulates the following sequence of events in the silicotic lung: silica fragments are transported into pulmonary

tissue spaces by phagocytes and come to rest in small collections throughout the pulmonary lymphatic apparatus; in course of time the phagocyte disintegrates liberating its load of siliceous dust into a connective tissue space; slow chemical change of the silica takes place with the production of a poisonous derivative which produces a local injury; slow proliferation of fibrous tissue is the response to this injury; the nodular morphology of the lesion is explained on the grounds that the dust, in the first instance, tends to collect in spherical foci.

Gardner's (53) dusting experiments have gone a long way to support the silica specificity theory. By causing rabbits and guinea pigs to inhale quartz dust over long periods measurable in years he induced a fibrous nodulation of the lungs comparable to that seen in the human disease and on the basis of this work is generally credited with proving that silicosis can be experimentally reproduced through the action of dust alone. To some morphologists it may seem as if experimentalists in general had taken unwarranted liberties with the generally accepted meaning of the term "silicotic nodule." But if there be any doubt as to the interpretation of the Gardner lesions, then that doubt is likely to be accentuated in respect of other experimental studies (66 a, 113 a, 133 a, 33) which purport to show reproduction of the silicotic nodule. The late Prof. E. H. Kettle, himself a veteran experimentalist in the field of pneumoconiosis, said (78) "Gardner has realised that silicosis is a chronic disease which takes years to develop. He has, therefore, exposed his animals to dusty atmospheres for eight hours a day over periods of two to three years and he has obtained most important results. But most other workers have been content with a total dust exposure of twenty-four to thirty-six hours, and I am not convinced that they have ever produced specific fibrosis; certainly they have produced nothing comparable with what occurs in man."

Whatever may be the merits of the silica specificity hypothesis there are certain important features of silicosis which it apparently does not explain, namely, 1, the great difficulty in reproducing the characteristic lesion in animals. In spite of numerous experiments there is no evidence, for example, to show that silica injected subcutaneously or intraperitoneally ever gives rise to nodular fibrosis comparable to that seen in the human disease. 2. The sporadic incidence of the disease. Watkins-Pitchford (164) points out "The mistake must not be made of supposing that all or even a majority of the miners will develop silicosis. It is only about 3 per cent of those who have worked for ten years or

longer who annually become silicotic in any form." Policard (122), on the basis of a wide experience of silicosis in France, also emphasizes this point.

Sometimes one has the opportunity of examining the lungs of individuals who have worked for years in phthisis-producing dust and have not developed silicosis. The present writers have been much impressed with this apparent immunity to silicosis amongst gold miners in Northern Ontario where silicosis is not uncommon. We have had the opportunity, through the kindness of Dr. J. G. Cunningham, of examining the lungs of several long term miners, who had survived the silica hazard for years and outlived silicotic work-mates of a similar age, to die eventually from some other cause. In such cases, as Mavrogardato (109) has pointed out, one invariably finds large amounts of particulate silica in the pulmonary tissues and one knows that it must have been there for years without provoking any appreciable reaction. This apparent immunity to silica, which must be regarded as of widespread occurrence, is difficult to reconcile with the theory that silica per se is a poisonous substance.

Haldane (60 a), well aware of this common immunity to silica, explained it on his well-known theory of adulterant dusts which postulated that certain relatively innocuous dusts (shale, clay, coal) may forestall the evil effects of silica if inhaled at the same time. Numerous attempts have been made to identify the hypothetical antidote which Haldane visualised (Mavrogardato, 109, 110; Carleton, 20; Haynes, 64; Hefferman, 65 a) but the results have been equivocal (Kettle, 80). Recent work in Sir Frederick Banting's (33) department in Toronto has given promise of allaying the pathogenicity of silica by reducing its solubility through the admixture of aluminium dust, but this conjecture awaits more crucial test. To quote Kettle (78) again "We must have a base line to work on, and in these experiments the base line must be the production of characteristic silicosis in animals. In the absence of this we are not justified in claiming experimental knowledge of how silicosis may be modified by adulterant dusts."

2. *The infective theory* of origin of the silicotic nodule finds its chief support in the fact that silicosis is so often accompanied by tuberculosis and in the fact that the silicotic nodule morphologically resembles the fibroid tubercle.

Towards the end of his long experience of silicosis in the Transvaal, Watkins-Pitchford (164) concluded "that in practically all instances there are two factors pre-requisite to the development of silicosis in its

usual form: 1, the condition of latent silicosis, and 2, the access of a tuberculous infection"; and further that "The pathology of almost all cases of what we term simple silicosis now appearing on the Rand is fundamentally the same as that of tuberculosis with silicosis; the development of what appears to be a condition of simple silicosis in these days, is probably a pathologically unique result of a tuberculous infection in a latently silicotic individual."

Kettle's (78) viewpoint is expressed thus "It is true that dust and dust alone may produce disability and death . . . but under modern conditions dust disease, or silicosis, really spells tuberculosis," and again (77) "Pure or simple silicosis is rarely of clinical significance; nearly always silicosis is associated with tuberculosis and my experience leads me to place increasing importance on the infective factor. . . . There has often seemed to me strong evidence that the apparently pneumoconiotic lesions were really infective from the beginning."

Policard (122) suggests that while pure silicosis is a theoretical possibility, it is rarely a practical reality—"En fait, dans l'immense majorité des cas, si non dans tous, le poumon silicotique est toujours aussi un poumon tuberculeux"; and Nicaud (120) writes "On peut donc vraiment se demander si la silicose a une existence propre et si les cas rapportés sous ce nom ne sont pas de simples modalités de tuberculose fibreuse". Rist et al. (132) also comment, "Il devient aussi de plus en plus évident que l'inhalation de poussières siliceuses ne suffit pas à elle seule à créer la silicose . . . c'est que l'intervention d'un autre facteur pathogène est nécessaire. Ce facteur est le plus souvent représenté par la tuberculose, et parfois sans doute par les simples séquelles de tuberculose."

Kettle and Archer (82) have drawn attention to the difficulty sometimes encountered in distinguishing between silicotic nodulation and fibroid tubercles while Nicaud (120) has done much to show a histological similarity between the two.

Kettle (77) by combining dead tubercle bacilli with siliceous dust produced lesions in guinea pigs' lungs comparable to the human silicotic nodule.

Policard and his associates hold strongly to the infective theory. They report inability to duplicate Gardner's experimental reproduction of silicotic nodulation by the action of dust alone. In Policard's prolonged and careful experiments (127), silica per se produced only relatively slight changes, but with the addition of tubercle bacilli (B.C.G.) a result, regarded as comparable to human silicosis, was obtained. Policard regards the silica as having a specific effect which only becomes

apparent in the event of subsequent tuberculous infection. In other words, only on the basis of a preëxisting "silica-granuloma" will the tubercle bacillus give rise to the characteristic fibrous nodule. He believes the silica undergoes some chemical change to produce this effect.

Not without impressive evidence does Policard (122) conclude his treatise on the "Mode of Formation of the Silicotic Nodule" thus: "Le nodule silicotique typique, celui qu'on envisage souvent comme 'pur', ne serait pas autre chose qu'un foyer tuberculeux cicatrisé et guéri, parce qu'il s'est formé au niveau d'un amas de particules siliceuses. La silicose serait une tuberculose pulmonaire devenue fibreuse sous l'influence et au niveau des particules siliceuses. Le nodule silicotique serait une lésion bacillaire développée au niveau d'un amas de poussières siliceuses et devenue tout à fait fibreuse sous leur influence."

Rist, Vallery-Radot and their associates (132) lend the weight of their authority to the view that the silicotic nodule is probably a modified tubercle.

There is of course unanimous agreement that if the silicotic nodule is to be construed as tuberculous then: 1, it represents an anomalous manifestation of tuberculous infection; 2, siliceous dust plays an essential rôle in its development; and 3, silica must modify the tuberculous process in a twofold manner, viz., by offering unusual impetus to fibrosis of the lesions, while at the same time facilitating the progress and spread of the infection.

The infective theory has the advantage of explaining 1, the sporadic incidence of silicosis; 2, the nodular morphology and the distribution of the essential lesion (see Nicaud, 120); 3, the close association of silicosis with tuberculosis; and 4, the difficulty of reproducing the silicotic nodule in animals by the action of silica alone.

Perhaps the most serious objections levelled at the infective theory have been 1, the well-known clinical observation that full-blown cases of silicosis may evince none of the usual signs and symptoms of pulmonary tuberculosis, and 2, the frequent inability to demonstrate tubercle bacilli in silicotic nodules (see Simson and Strachan, 149).

XV. The influence of silica on the tubercle bacillus. How to explain the tuberculous affinity of the dust-ridden lung has been the subject of intensive study for years. One of the first things to be investigated was the influence of various forms of particulate silica and silicates on the growth of tubercle bacilli in vitro, and in general negative results

seem to have been obtained (Kettle, 78) though Price (129) reported some acceleration of growth with sodium silicate.

The crucial experiments of Gye and Kettle (57) showed a selective localisation and pullulation of tubercle bacilli in subcutaneous silica-lesions in mice, from which these authors suggested that the necrosis induced by silica afforded a protective pabulum favourable to proliferation of the tubercle bacillus. Later, Kettle (81) was able to show that the active growth of the bacilli did not depend merely on the necrosis of the tissue, but was due to the actual presence of the silica. Cummins and Weatherall (29) repeated and confirmed this work but found that the tubercle bacilli tended to disappear in time from the silica lesions, without affecting the survival of the animals.

Vorwald and Landau, (162) working in Doctor Gardner's laboratory at Saranac Lake, also undertook a repetition of the Gye and Kettle experiments. Using white mice they injected various mineral dusts beneath the skin of the abdominal wall. The animals were then infected intravenously with living tubercle bacilli and were killed at different periods after infection. When the subcutaneous dust lesions were examined histologically for the presence of tubercle bacilli it was found that those lesions produced by free silica were more often positive and contained more bacilli than those caused by silicates and non-siliceous dusts. This work, in the main, confirms Kettle's (81) results, but with one reservation, namely, that while silica lesions seem to favour tubercle localisation more than do the lesions of other dusts, yet the latter are not uniformly inimical to the tubercle bacillus. Silicates like sericite, talc and sillimanite and even some non-siliceous powders like fluorspar and aluminium oxide were not without some effect which seemed to favour tubercle localisation.

Gardner has produced reactivation of healing tubercles in guinea pigs' lungs by experimental inhalation of marble, granite, carborundum and quartz dust (51). He also showed that the combined effect of tuberculous infection plus any one of these dusts produced more fibrosis than either agent acting alone (52).

Of special significance are those experiments of Gardner (55) in which he showed that the native resistance of guinea pigs to an attenuated strain of tubercle bacilli (Saranac Lake R1 strain) was altered by silica. In the ordinary course of events guinea pigs infected by inhalation with this organism recover completely, but when dusted with quartz the infection spreads and progresses to fatal proportions. This change is

not due to an increased virulence of the organism, for subinoculations produce merely the characteristic attenuated infection. Gardner believes the silica alters the defence mechanism of the phagocytes by injuring them and perverting their bactericidal activities.

Thus, the laboratory evidence as well as clinical experience all goes to emphasize a notable adjuvant effect of siliceous dust in respect of tuberculous infection. This effect seems to depend upon some intermediary interaction between the dust and the tissues rather than upon the dust itself. Whether the essential intermediary change is humoral as Cummins (30) suggests or cellular as Kettle (78) thought, whether it is lymph stasis, necrosis, fibrosis or incapacitation of phagocytes, remains to be proven, but the available evidence favours the view that the silica-vulnerability of the phagocyte is an important link.

In conclusion it may be said that there has been and there still is much discussion about the relative harmfulness of various siliceous dusts (9). The general tendency has been to regard crystalline silica as the sine qua non of pathogenicity in harmful dusts, but there is reason to believe that some of the silicates may have a like effect. Much work remains to be done before we can satisfactorily gauge the harmfulness of various silicates, but it seems to us not unlikely that future simplification of the silicosis problem may turn more upon further elucidation of the alliance between siliceous dust and the tubercle bacillus than upon assessing the relative pathogenicity per se of various compounds of silicon. There is also the broad and open question of what influence silica may have upon infective organisms other than the tubercle bacillus and to what extent they may be linked with silica in the production of dust fibrosis. Our experience has left us with the impression that what we now know for certain about the pathogenicity of silica is perhaps not very much compared to what we have yet to learn.

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THE RÔLE OF INFLAMMATION IN IMMUNITY[1]

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The phenomenon of inflammation, as manifested in the vertebrate kingdom, may properly be regarded as the physical basis of infectious processes. The significant and obvious relationship of bacterial infection to inflammation must not, however, overshadow the broader implications of the inflammatory reaction. It is to be remembered that essentially the manifestation of inflammation tends to develop along a uniform pattern irrespective of the irritant. Foreign substances, whether viable or non-viable, in contact with normal tissue will induce an inflammatory reaction, the intensity of which may vary from a barely visible hyperemia to an intense suppurative process. For obvious reasons, however, the interest of the microbiologist in problems of tissue injury has primarily been focussed on bacterial infection.

Inflammation may be broadly defined as the complex vascular, lymphatic, and local tissue reaction elicited in higher animals by the presence of microörganisms or of non-viable irritants. It represents a basic or elemental reaction to injury whereby the deleterious agent tends to be localized and ultimately destroyed. The inflammatory reaction may be truly regarded as an immunological mechanism of definite significance.

The purpose of this review is to summarize the recent evidences relating inflammation to the broader aspects of immunity. This, in the last analysis, is doubtless its most significant rôle in bodily processes. Some of the various mechanisms involved in the development of the inflammatory reaction will be pointed out, paying particular attention to the orderly sequences that occur from the initial introduction of an irritant until its ultimate disposal. The importance of inflammation

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as a significant factor regulating bacterial invasiveness in the non-immune as well as in the immune animal will likewise be considered.

I. *Development of the Inflammatory Reaction.* 1. *The vascular capillaries in inflammation.* The inflammatory reaction is initiated by an alteration in the local fluid exchange. This is primarily referable to a basic disturbance in the normal filtration equilibrium between the small vascular channels, the lymphatics, and the so-called intercellular fluid.

The most satisfactory hypothesis that explains filtration between blood and tissue fluid was offered by Starling (1896). The concept was subsequently verified by the measurements of Landis (1926, 1927 a, b). In brief, Landis concluded that the gradient of pressure along the course of the minute vessels favors filtration at the arteriolar portion of the capillary and reabsorption at its distal part. The rate of fluid passage appears to depend on the balance between the capillary pressure and the osmotic pressure of the plasma proteins. These original conclusions were based on an assumption that the endothelial lining resembled an inert membrane and that it was essentially impermeable to the outward passage of proteins. This concept was somewhat modified by Drinker and his collaborators who demonstrated that the endothelial wall of capillaries was not wholly impermeable to the leakage of proteins. An appreciable concentration of proteins was recovered from the lymph which reduced markedly the effective osmotic pressure of the plasma colloids (Churchill, Hakazawa, and Drinker, 1927; Drinker and Field, 1933).

In the last few years the importance of the capillary filtration theory of Starling, revived by Schade (1927) and verified by the ingenious measurements of Landis (1926, 1927 a, b), has been critically scrutinized in view of the findings of Rous and his collaborators (1930). These investigators injected intravenously some poorly diffusible dyes into frogs and also into mammals (guinea pig, rabbits, and mice). The dye was observed to escape primarily from the distal part of the capillary and from the small venules, thus giving rise to a mounting gradient of permeability.

The gradient was observed to be independent of various factors, in cluding hydrostatic pressure (McMaster, Hudack, and Rous, 1932), osmotic pressure (Smith and Dick, 1932), nervous section, hemorrhage, or cessation of circulation. Rous concluded that the permeability gradient was most likely referable to structural differentiation of the capillary wall rather than to any functional alteration. To Rous the mounting permeability gradient represents an adaptive mechanism for the equalization of opportunity in the distribution of diffusible nutrients

to tissue. Through such a delicate regulatory mechanism a homeostatic state is effectively maintained in tissue, in accordance with Bernard's concept of the "milieu interne."

On the basis of his results Rous cast some doubt on the validity of the Starling hypothesis as an adequate explanation of fluid exchange. Rous and Smith (1931) failed to confirm Landis' observation on the passage of trypan red (vital red HR) through the capillary wall of the frog mesentery. Landis (1927b) reported that this dye frequently escaped from an individual capillary "in greater amount outside the arterial portion of the vessel." In view of the elevated pressure at the arterial end of the capillary, this was interpreted as additional evidence supporting the pressure gradient hypothesis. Rous and Smith correctly pointed out the possibility of capillary injury and distortion as a result of prolonged exposure to physiological solutions. The findings of the latter investigators were consistent. The dye in question, as well as trypan blue and Chicago blue 6B, escaped first from the distal portion of the capillaries and the smallest venules. Landis has recently criticized the conclusions of Rous and Smith because of the large amounts of dye used and for their failure to measure capillary pressure (1934). It is difficult to see how the amount of dye injected would be the conditioning factor in inducing the formation of the characteristic permeability gradient. Landis (1934) insists that observations made with a relatively poorly diffusible dye should not be stressed too far in the evaluation of their results: "The gradient is therefore demonstrable only with dyes whose diffusibility is much less than that of the blood constituents concerned in nutrition. To what extent observations with poorly diffusible dyes can justifiably be applied to the highly diffusible constituents of blood is still a matter of inference and conjecture." Peters (1933, 1935) agrees that the Rous permeability gradient may be concerned in certain tissues with the equalizing of opportunities in distribution of nutrient material of large molecular dimensions. But according to him this fact in itself alters in no way the Starling-Landis hypothesis which is still considered the most reasonable explanation for the local fluid exchange of water and electrolytes through the capillary wall.

As stated above, the introduction of a chemical or bacterial irritant into normal vertebrate tissue is followed by definite disturbances in the filtration equilibrium. There is local increase in the passage of plasma as evidenced by the edema which may develop fairly early. Both the capillary pressure and the mounting permeability gradients are so

profoundly altered as to favor enhanced fluid accumulation in the affected tissue. The principal forces at play in promoting these changes may be listed as follows:

1. Increased capillary pressure accompanying local vasodilatation.
2. Increase in the actual number of functioning capillary channels.
3. Increase in capillary permeability. The initial constriction of the small vessels which is frequently encountered will not be discussed here owing to the lack of exact data on the mechanism and significance of this phenomenon.

Krogh (1922) assumed that the increased filtration through the capillary wall following the use of urethane was referable to the stretched endothelium of the dilated capillary. Landis (1927 b) demonstrated by intravascular injection of dyes that there was no measurable increase in permeability with dilatation of the capillaries. The passage of fluid seemed to be primarily referable to the level of intracapillary pressure. This investigator regards the effect of urethane as the result of direct injury to the endothelial lining accompanied by an increased capillary pressure.

The available data indicate that capillary dilatation in hyperemia or inflammation is accompanied by increased capillary pressure (Landis, 1931). This elevated pressure constitutes a definite factor that favors the outward passage of fluid. Landis concluded that the rise in capillary pressure in hyperemic conditions was referable to arteriolar dilatation. There was no indication in his experiments of any increased venous pressure to account for the augmentation in the intracapillary level. Hyperemia was induced, for instance, by placing a crystal of silver nitrate on the skin of the web of a frog. Within 1 to 3 minutes there was a rapid rise in capillary pressure which returned, as a rule, to the resting level within 7 to 20 minutes. The average rise in pressure varied between 19.5 cm. of water to 16.5 cm. depending on whether measurements were made in arteriolar or venous capillaries. Landis believes that this transient rise in pressure is referable to a stimulation of the axon reflex which causes arteriolar dilatation with accompanying increase in capillary pressure. It is obvious that an increase in pressure within the capillaries would be significant in promoting filtration through the endothelial wall.

Probably an even more striking factor favoring increased seepage into the extracapillary spaces of an inflamed area is an actual change in the endothelial lining which increases its permeability or capacity for permitting the passage of fluids. Cohnheim called attention to this

phenomenon about fifty years ago. Landis (1927 b) pointed out that capillaries injured by alcohol and mercuric chloride appear to be permeable to the plasma colloids and approximately seven times more permeable to fluids than is the normal capillary wall.

The extent of injury to the capillary wall can be demonstrated in the early phase of the inflammatory reaction by a variety of means. For instance, graphite particles that normally fail to pass through the endothelial membrane readily do so in the early stage of an acute inflammatory process (Menkin, 1931 d). Another convenient method of demonstrating the alteration in the permeability of the endothelial wall in injury is by the use of intravenously injected dyes. Trypan blue and other dyes introduced into the circulating blood promptly accumulate in an inflamed area. The localization of vital dyes in areas of inflammation has been demonstrated by several investigators. MacCurdy and Evans (1912) pointed out that the normal brain and cord always remain free from dye injected intravenously but that areas of damage, such as softening or inflammation, become deeply stained. Bowman, Winternitz, and Evans (1912) found that trypan blue injected intravenously stains tubercles in experimental tuberculosis. Subsequently Winternitz and Hirshfelder (1913) demonstrated that this dye when injected intravenously in experimental lobar pneumonia stains the consolidated area of lung selectively. Lewis (1916) found that if the cornea of a rabbit is inoculated with a living culture of tubercle bacilli, a progressive lesion results characterized by an intense congestion of the conjunctiva. If the animal receives an intravenous injection of trypan red 24 hours or more after such inoculation, the fluid in the anterior chamber of the inoculated eye always becomes colored. Precisely similar results were obtained when abrin was administered in the conjunctiva as an inflammatory irritant. A few years ago McClellan and Goodpasture (1923) showed that trypan blue accumulates in lesions of herpetic encephalitis in the rabbit's brain, the injured areas presenting a striking color against the quite unstained healthy brain tissue. Siengalewicz (1925) pointed out that general damage to the nervous tissue, such as poisoning with carbon monoxide or with salvarsan, is followed by marked staining of the damaged areas by trypan blue. Ramsdell (1928) injected trypan blue into the veins of rabbits and guinea pigs previously treated with foreign serum and found that injection of the same serum into the skin of the ear immediately caused local infiltration of the dye into the adjacent tissue. She regarded the infiltration of the dye as an indicator of edematous changes resulting from toxic injury to the capillary endothelium.

Okuneff (1924) found that a thermal irritant favors the passage of vital stains from the blood stream into the area heated. Kusnetzowsky (1925) also observed that the local application to the skin of an irritant, such as heat or mustard oil, causes an accumulation of trypan blue in the inflamed area when the dye has previously been injected into the blood stream. Menkin (1929) demonstrated that trypan blue injected into the circulating blood enters the site of inflammation rapidly and is fixed there, so that the tissues are deeply stained. Furthermore, trypan blue injected directly into the site of inflammation in the subcutaneous tissue or in the peritoneal cavity is fixed in the inflamed area and fails to reach the regional lymphatic nodes. This work clearly showed that there is not merely a rapid accumulation of the dye from the blood stream into an acutely inflamed area, but also that the dye is held in such an area and is unable to drain readily away through the regional lymphatics.

The accumulation in inflamed tissue of a dye that has been introduced into the blood stream is doubtless primarily the result of an increased permeability of the capillaries which is part of the inflammatory reaction. Menkin and Menkin (1930) were able to secure quantitative data by studying directly the change in the concentration of the dye in the blood stream both in the inflamed and in the normal mesentery of the frog. They found that, with the irritants employed, the permeability of the capillaries in the injured area was almost doubled.

Sufficient evidences have been adduced indicating that increased filtration in inflammation is primarily the result of an alteration in the permeability of the capillary wall. This, in turn, is reinforced to some extent by a transient and initial increased capillary pressure.

2. *Mechanism of increased capillary permeability in inflammation.* The precise mechanism involved in the initial augmentation of capillary permeability in injury is obviously of considerable importance for an adequate understanding of the subsequent sequences in the development of an inflammatory reaction. In 1923 Ebbecke postulated that a substance is formed by irritated epithelium which on diffusing to the cutis dilates the capillaries and smallest arterioles. Subsequently Lewis (1927) postulated the development of a type reaction primarily referable to a chemical H substance liberated from injured tissue. The H substance is presumably histamine or a substance having similar properties and therefore resembling it closely. According to Lewis and Grant (1924) the type reaction elicited by the cutaneous injection of histamine manifests itself in three ways: *a*, a local vasodilatation of capillaries, venules, and arterioles by direct action; *b*, a widespread

dilatation of outlying arterioles resulting from a local reflex; and *c*, an increase in the permeability of the minute vessels by direct action. This type reaction leads to local edema of the skin.

The conclusions of Lewis and Grant (1924) appear to be based largely on an analogy of the type reaction obtained by histamine with that of a variety of other injurious agents. When more direct tests were performed on the effect of the skin wheal fluid on the contraction of the guinea-pig uterus, they were unable to obtain any evidence that histamine was liberated in larger quantities from injured tissue than was found in normal plasma.

Krogh (1929) accepts unreservedly the view first foreshadowed by the work of Ebbecke (1923), and later chiefly sponsored by Lewis, that under certain stimuli the tissue cells will liberate substances having a dilator effect on capillaries; but at the same time he finds it impossible to assume that in all cases the action is referable to a single chemical H substance. For this reason Krogh postulates the possibility of two effective substances liberated from injured cells: a diffusible factor closely related to histamine, if not histamine itself; and an H colloid substance which is probably less diffusible. Rous and Gilding (1930) raise considerable doubt concerning the validity of a hypothesis which refers all local vasodilatation to the action of a single chemical substance liberated within tissues. They point out that the vascular contraction in Bier's spots prevails over the local reddening induced by mechanical injury, whereas it is without effect upon the local vasodilatation induced by cutaneous injection of histamine. More recently Goldschmidt and McGlone (1934) have studied the failure of a reactive hyperemia occurring with arrested circulation in an oxygen atmosphere. They have compared their findings with the histamine reaction in a similar oxygenated and ischemic environment. Their observations on the human forearm seem incompatible with the view that the vasodilatation responsible for reactive hyperemia is due to an H substance identical with histamine. By comparing the reactions of the dilated minute skin vessels to adrenaline puncture and to Bier's spots, Percival and Scott (1931) have been unable to conclude that the vascular reactions in exfoliative dermatitis, ultra-violet erythema, oil of mustard erythema, psoriasis, dermatitis venenata, and tinea corporis (ring-worm) were due to a histamine-like substance or histamine itself. That histamine is not always the active substance responsible for the increased capillary permeability in injury is indicated by the observations of Grant and Jones (1929) on the frog. This substance causes no dilatation of the

frog's blood vessels either when applied locally or when given intravenously. They express the belief that in the frog the active substance is a base of the histidine-arginine fraction which is responsible for the vascular reaction to injury. The contention raised by Rous and Gilding has been reinvestigated by Wayne (1931) who arrives at the conclusion that the vascular reactions resulting from injury to the skin are due to a liberated substance, either histamine or some substance closely allied to it. This worker answers the objection of Rous and Gilding by maintaining that the vasoconstrictor effect of Bier's spots can overcome the slight local vasodilatation produced by histamine, provided the latter is introduced electrophoretically into the skin in a diffuse manner and in low concentrations. The vasoconstriction will, however, not prevail over a more intense local vasodilatation induced by greater concentrations of histamine. He therefore concludes that the vessels of the skin, under the influence of either histamine or the H substance liberated by injury, behave similarly towards Bier's spots when the two are distributed in a comparable way throughout the tissue spaces.

A study was undertaken by Menkin (1936 a) in order to determine whether one or more substances could be obtained from inflammatory exudates which, when introduced into normal cutaneous tissue, would induce local vasodilatation and an increase in the permeability of the capillary wall. Furthermore, the properties of the active fractions which have been obtained from inflammatory exudates have been compared with histamine in an endeavor to test Lewis' hypothesis. In brief, the writer's observations indicate that a diffusible crystalline material capable of increasing capillary permeability is present in various types of inflammatory exudates. Its liberation and presence in exudates offers a reasonable explanation for the mechanism of increased permeability of small vessels in injured tissue. By appropriate tests this active principle has been shown to lack the properties characteristic of histamine (Menkin, 1936 a, 1937 b, 1938 a). No concrete evidence was obtained to support Lewis' view of a detectable histamine-like substance in exudates or in their partially purified fractions. The possibility, however, that histamine exists in exudates is not wholly dispelled by the available evidence on the presence of the permeability factor (Menkin, 1936 a; cf. Loos, 1931); but the primary importance of histamine in explaining the mechanism of increased capillary permeability in inflammation is open to serious doubt. The finding by Feldberg and his associates (1937) that perfusion of the lung by snake venoms, staphylococcus toxin, or peptone, induces the liberation of

proteins and of histamine is no demonstration that the latter substance is primarily involved in the mechanism of enhancement of capillary permeability during injury. Countless substances are probably liberated from a site of inflammation, but whether many of these participate as important factors in directly determining increased capillary permeability is another matter. The case of histamine has not been satisfactorily proven. The permeability factor recovered from an exudate, described and isolated by Menkin (1936 a, 1938 a), displays none of the properties of histamine. When histamine and the permeability factor were compared with regard to their respective properties, the following observations were made: *a*, upon the intravenous injection of dye the local staining pattern differed with each of these two substances; *b*, in contrast to the usual effect elicited by histamine, the permeability factor failed to cause a contraction of the isolated intestine; *c*, following treatment with the permeability factor the intestinal segment exhibited no refractory state; the usual contractile effect elicited by histamine occurred; *d*, evidence was obtained of the antagonistic action of histamine to the active principle when mixed *in vitro* and then tested on the capillary wall; *e*, the specific Zimmermann color reaction for histamine was negative when tested with the permeability factor; *f*, a rapid chemotactic effect was induced by the permeability factor; histamine apparently does not cause leukocytic migration; *g*, the permeability factor failed to depress blood pressure in the cat in the manner characteristic of histamine. Recently Abramson and his collaborators (1938) have further confirmed, by an iontophoretic method, the earlier conclusions of the writer that histamine is probably not responsible for the production of hyperaemic wheals.

The method first used by the writer to determine the effect of the permeability factor on the endothelial wall consisted of injecting the cell-free exudate intracutaneously in rabbits. This was almost immediately followed by an intravenous injection of trypan blue. The extent of accumulation of the dye in the treated cutaneous area served as a gauge of capillary permeability. Subsequently the permeability factor was purified by treating the exudate with pyridine followed by acetone (Menkin, 1938 a). After separation of the protein fractions further purification was obtained by prolonged extraction with butyl alcohol or by subjecting the acetone or butyl supernatant fractions to low temperature (−20°C.). The latter favors spontaneous separation of the active principle. The purified material is a crystalline doubly refractive nitrogenous substance, capable of inducing increased capillary permeability in dilution of 1:100,000.

The permeability factor is evidently not a protein, yet it contains amino and carboxyl groups. It gives a positive test for the presence of an indole nucleus in its structure (Adamkiewicz test). The active material is thermostable. It is dialyzable; and it is precipitated by concentrated ammonium sulphate. The present evidence indicates that it is an intermediary product of protein metabolism probably belonging to the group of relatively simple polypeptides.[2] In view of its chemotactic property the writer has tentatively, for the sake of convenience, named this active substance *leukotaxine*. The liberation of this substance in injured tissue may thus be referable to an interference with normal protein catabolism occasioned by the presence of an irritant.

Duran-Reynals (1929) described a spreading factor present in extracts of certain tissues, particularly the testis. This agent facilitates the extension of India ink from the site of its cutaneous inoculation. He referred the phenomenon to an enhancement of tissue permeability by the organ extract employed. Recent unpublished observations indicate that the crystalline material recovered from exudates (leukotaxine) is probably unrelated to this spreading factor. The Duran-Reynals substance is relatively thermolabile and non-dialyzable, whereas the permeability factor in exudates is both thermostable and dialyzable. Preliminary studies have shown that in testicular extracts both factors may be present, and that the spreading factor of Duran-Reynals may be dissociated from a heat stable permeability factor which is apparently similar to, if not identical with, the one recovered from inflammatory exudates (Menkin, 1937 b).

The observations brought forth above suggest that the increased capillary filtration of inflammatory conditions is primarily referable to: 1, a transient elevation in capillary pressure; 2, an increase in capillary permeability.

Do reactions on the part of nerves play a rôle in inducing these capillary changes? This question as yet remains more or less unanswered. Cohnheim (1889) maintained that the primary *modus operandi* accounting for capillary filtration was a molecular alteration of the endothelial wall presumably effected directly by the irritating substance. The result is a prompt increase in capillary permeability. The vasomotor nerves, according to Cohnheim, play no part in the reaction. The reasons presented for arriving at this conclusion are perhaps open to doubt, particularly as Cohnheim seemed to be of the opinion that

[2] Available physiological evidences indicate that *leukotaxine* is neither acetylcholine nor an adenylic compound (Menkin, unpublished observations).

permeability changes are detectable only some hours after the application of an irritant. In this connection Menkin (1932 b) has demonstrated an increase in capillary filtration as early as two minutes after the introduction of certain irritants.

Bruce (1910) studied the inflammation produced in the conjunctiva of rabbits by a drop of mustard oil. He reported that the maximal capillary dilatation could be greatly diminished by local anesthesia with cocaine. Furthermore the dilatation fails to appear when the cut nerves are allowed to degenerate. He therefore concluded that the effects must be due to a local nervous mechanism. He referred the mechanism to an axon reflex located in the sensory fibers. These results have essentially been confirmed by Bardy (1915) and by Breslauer (1919).

Krogh (1920) observed that the application of iodine to the frog's tongue produced a violent contraction of the underlying muscles. The capillaries of the mucous membrane strongly dilated and the dilatation extended for a considerable distance. After the application of cocaine to paralyze the sensory nerves and the nerve endings, a drop of iodine failed to elicit the usual effect. When iodine was applied to the mucous membrane after section and degeneration of the lingual nerves, the affected capillaries became partially dilated over a localized area. Krogh (1922) concluded that, at least for certain substances, nervous reactions play some part in the initial inflammatory symptoms. The nerves which are responsible for the reaction are most likely sensory fibers that induce dilatation through local axon reflexes.

Ricker and Regendanz (1921), after studying the local action of a number of substances on the pancreas and conjunctiva of the rabbit, reject the views of Cohnheim in regard to the rôle of the vascular nervous system in inflammation. These investigators assume, with, however, only meager evidence, an intricate arrangement of vasomotor nerves consisting of constrictor and dilator fibers supplying both arteries and capillaries. They contend that tissue alterations caused by the presence of an irritant are, in the last analysis, due to local circulatory disturbances arising through stimulation of involved vasomotor nerves.

More recently the studies of Inutsuka (1928) confirmed the earlier observations of Samuel (1890) and the later ones of Meltzer and Meltzer (1903 a, b) on the effect of sectioning the cervical or sympathetic nerves in enhancing exudation. This investigator demonstrated that excision of sympathetic ganglia induces marked exudation.

The stimulation of the peripheral ends of sensory nerves induces vasodilatation of skin vessels (antidromic impulses). Lewis and Marvin (1928) and Ungar et al. (1936) are of the opinion that such stimulation

favors the liberation of histamine in the skin. Wybauw (1936) on the other hand believes that antidromic impulses release acetylcholine. The available data fail to settle this controversial point. At any rate there seems to be no strong proof that either one of these substances, even though perhaps released, is necessarily concerned with inducing the cutaneous vascular dilatation which results from stimulation of sensory nerves.

The foregoing observations indicate that local nervous reflexes probably play a rôle in modifying the initial development of the inflammatory reaction. The experiments of Landis (1931), cited previously, pointed out quite definitely that the transient increase in capillary pressure following the application of an irritant was apparently referable to a local axon reflex. It is conceivable, in view of these facts, that nerves play a rôle in inflammation merely by inducing a temporary increase in capillary pressure thereby favoring outward filtration. On the other hand, there is little doubt that, as pointed out by Cohnheim, an acute inflammation may develop in the absence of all central nervous influences. The persistent and far more significant phenomenon of increased endothelial permeability in inflammatory processes appears to be primarily referable to the liberation of the permeability factor (leukotaxine) by injured tissue. Whether the release of this active crystalline substance is any way related to initial local axon reflexes; or whether, on the contrary, its presence plays some definite part in the development of such reflexes still remains to be determined.

3. *The migration of leukocytes into inflamed tissue.* The preceding sections have dealt with capillary changes brought about by the presence of an irritant. The increase in capillary permeability allows the outward passage of plasma proteins. The early formation of fibrin in the inflamed area plays an important rôle in immunity by circumscribing the irritant. This phase will be discussed at length in a subsequent section. The importance of the emigration of leukocytes into injured tissue was demonstrated in the middle of the nineteenth century by Cohnheim (1867). The significance of phagocytosis in the disposal of an irritant was stressed primarily by the classical studies of Metchnikoff (1892). This phase of the field has been already discussed at length, and lack of space prevents any further reference to it (cf. Mudd, McCutcheon, and Lucké, 1934).

The attraction of leukocytes to a site of injury is an old concept dating back to the work of Leber in 1879. This investigator found a chemotactic substance in the bodies of dead cultures of staphylococci. This substance, which he called *phlogosin*, was found capable of attracting

leukocytes in tissues. The early work of Massart and Bordet indicated the chemotactic influence exerted by the products of leukocytic disintegration (1891). Gabritchevsky (1890) studied a number of substances, such as lactic acid, which actually have a repellent action on white cells (the so-called "negative chemotaxis"). Dead or living cultures of bacteria, on the other hand, were found to incite strong positive chemotaxis. Buchner (1890) reported the finding of a protein from the Friedlander organism, capable of exerting a strong chemotactic influence. He found that glycine and leucine were definitely chemotactic whereas tyrosine and trimethylamine failed to attract leukocytes. Wolf studied the phenomenon of chemotaxis *in vitro* (1921). She found that the calcium ion was the only inorganic ion which *per se* was positively chemotactic. She reported that, to a certain extent, all amino acids and amines are likewise positively chemotactic.

Grand and Chambers (1937) recently reported that mechanically injured tissues liberate thermolabile substances which are positively chemotactic for polymorphonuclear leukocytes. McCutcheon and Dixon studied chemotropism of polymorphonuclear leukocytes *in vitro*. Their results led them to conclude that the chemotropic response is one of direction only. The rate of motion depends on other factors such as the osmotic pressure of the medium, temperature, or the internal condition of the cell (1936). These workers found that, whereas polymorphonuclear leukocytes were strongly attracted by staphylococci or tubercle bacilli, lymphocytes failed to exhibit any chemotropism (1935). Very careful observations on the emigration of leukocytes in the living animal (amphibian larvae and rabbits) were recorded by Clark and his collaborators (1920, 1936). In general these investigators noted that the emigration of leukocytes commences on the average about 2½ hours after the injection of the irritant (croton oil). The majority of the cells were polymorphonuclear leukocytes. Relatively fewer monocytes or lymphocytes migrated through the endothelial wall. Furthermore, the period of emigration lasted only several hours. After a while the endothelial wall seemed to undergo a reversal in consistency which prevented further migration. Strong chemotactic response by polymorphonuclear leukocytes was not necessarily accompanied by active phagocytosis on the part of these cells.

The mechanism of the migration of leukocytes to an area of injury has not yet been satisfactorily explained. Nordmann and Rüther (1930) believe that the phenomenon is dependent on a retardation of blood flow in an inflamed area. The hypothesis that migration of leukocytes is referable to changes in surface tension or that the difference in

potential between normal and injured tissue favors the wandering into injured tissue of negatively charged leukocytes is still in large part the result of inference (Abramson, 1927, 1933; Weden, 1933). There is as yet little direct experimental observation to substantiate these views. Grant and Wood (1928) reported that histamine fails to induce leukocytic migration. They, therefore, concluded that the release of histamine from injured tissue does not provide a complete explanation of the process of inflammation. Moon (1935) essentially confirmed these observations.

As described above, the writer recently isolated a crystalline nitrogenous substance from inflammatory exudates which is *per se* capable of inducing a marked increase in capillary permeability. The substance is concerned not merely with an alteration in the permeability of small vessels but also with the mechanism of leukocytic migration (Menkin, 1937 b, 1938 b). The introduction into the skin of rabbits of this active material induces an almost immediate increase in capillary permeability followed by rapid migration of polymorphonuclear leukocytes. Within 15 to 20 minutes the lumina of the small cutaneous vessels are crowded with these cells, many of which adhere to the endothelial lining. Within 30 to 40 minutes the extracapillary areas are seen to contain a considerable number of polymorphonuclear leukocytes that have migrated outward from the blood stream. This cellular reaction does not seem to be referable to the preliminary enhanced capillary permeability. Strong irritants, such as aleuronat or turpentine, although producing a prompt increase in the permeability of capillaries fail to induce a rapid chemotactic effect. Furthermore, this active substance, tentatively named *leukotaxine*, is *per se* chemotactic as indicated by *in vitro* tests, as well as by observations on the accumulation of white cells in glass capillary tubes containing the active material. The available evidence supports the view that the permeability factor and the chemotactic factor are referable to one and the same crystalline substance. Leukotaxine seems to belong to the group of relatively simple polypeptides (Menkin, 1938 a, b). It is conceivable, as mentioned above, that its formation from injured cells results from an interference with normal protein catabolism by the presence of an irritant. The basic sequences of the inflammatory reaction, namely, the initial increase in capillary permeability followed by leukocytic migration, are thus perhaps referable to a common denominator: the liberation of leukotaxine. Variation in type and in intensity of cellular infiltration may be in part a function of the local concentration of leukotaxine. There are doubtless other factors involved in the development of the inflammatory reaction. For in-

stance, fibrin formation and thrombosis of small vessels do not seem to be directly related to the presence of leukotaxine (Menkin, 1938b, d). Unpublished observations indicate that leukotaxine, in addition to altering the permeability of the small vessels and inducing rapid leukocytic migration, produces probably little tissue injury. The latter may possibly be due to one or several other factors that still remain to be unravelled. Furthermore, studies in progress indicate that the leukocytosis which frequently accompanies severe inflammatory conditions is apparently not referable to the liberation of leukotaxine. This substance seems to be concerned with the mechanism of migration without directly affecting the number of leukocytes in the circulating blood.

For many years it has been known that the cytologic sequence in acute inflammation is characterized in the earliest stages by active migration of polymorphonuclear leukocytes. After a time this is followed by infiltration of mononuclear phagocytes or macrophages.

Space does not permit a discussion of the various views concerning the origin of the macrophages in inflammation. Suffice it to say that some investigators, notably Möllendorff, believe that macrophages are derived *in situ* by amitotic division of fibrocytes (1927). Weatherford has obtained further evidence indicating that in the earliest phase of inflammation a conversion of fibrocytes into histiocytes can readily take place (1933). Maximow and Bloom on the contrary deny this contention (1935). Bloom believes that most of the mononuclear cells in inflammation migrate from the blood and are transformed lymphocytes and monocytes. Relatively few of the cells originate from the macrophages already present in the affected tissue. Clark and his collaborators (1936) recently called attention to the possibility of artefacts. Injured or degenerating polymorphonuclear cells often round up and may present the appearance of mononuclear cells, round cells, or lymphocytes.

In acute inflammation the polymorphonuclear cells that leave the circulating blood stream form the chief cellular constituents of the early exudate. The mononuclear phagocytes or macrophages increase in number in the later stages. This orderly cytologic sequence in the development of the inflammatory reaction was first pointed out by Borrel (1893) and then by Durham (1897) and Beattie (1903). Such a sequence seems to characterize the majority of acute inflammatory reactions caused by bacteria or by chemical irritants.

No adequate explanation had been offered for this fundamental process. The earlier studies of Müller (1888), of Opie (1905, 1910),

and later of Weiss (1927) indicate the presence of proteolytic enzymes in leukocytes. Leukoprotease, found in polymorphonuclear leukocytes, is active only in a neutral or slightly alkaline medium. Lymphoprotease recovered from mononuclear phagocytes is, on the other hand, active in an acid medium. These old observations suggested to the writer that there may be a parallelism between the concentration of hydrogen ions in the exudate and the pH which is optimal for the action of the intracellular enzyme of the predominating type of phagocyte. If this assumption were correct, one might expect in acutely inflamed areas the development of a local acidosis with the shift from polymorphonuclear to mononuclear phagocytes. Lord (1919) in his studies of the pneumonic lung had concluded that in the course of the disease an increase in concentration of hydrogen ion in the exudate probably occurs. Schade and his collaborators (1921) reported that pus from acute abscesses had a pH ranging from 5.95 to 6.50. Menkin studied the relation between the pH of the medium and the cytologic picture during the course of an acute inflammatory reaction (1934 a). The observations indicated that the predominance of polymorphonuclear leukocytes at the beginning of an inflammatory reaction is correlated with a pH of the exudate ranging between 7.4 to 7.25 or thereabouts. With the progress of the inflammation the pH may fall to a level of 6.9 or 6.8. At this stage the exudate is characterized by a preponderance of macrophages. The polymorphonuclear cells are found swollen, vacuolated, and appear degenerated. Below a pH of 6.8 or 6.7 all types of leukocytes are injured, and frank pus ensues. Observations indicate that there seems to exist a definite relation between the pH and the cytologic picture of an exudate. When the inflammatory reaction is mild the pH persists above 7.0, and the polymorphonuclear cell type predominates. Furthermore, Evans reported that leukocytes are sensitive to the action of acids (1931). The writer has recently been able to verify this observation (1938d). In brief, with a fairly reasonable degree of certainty, the cytologic picture of an exudate can be predicted from its hydrogen ion concentration and vice versa.[3] The data

[3] Steinberg and Dietz have recently questioned the repeated findings of a great number of investigators concerning the development of local acidosis in acutely inflamed areas (Arch. Path., 1938, **25**, 777). As indicated in detail elsewhere (1938d) by the writer, their contention may primarily be invalidated by a questionable technique in the measurement of pH and by their failure to correlate the leukocytic picture and pH on an identical sample of exudate. Menkin's conclusions have recently received further confirmation in the hands of Lurie (Amer. J. Path. 1937, **13**, 612).

suggest that the cytologic picture in inflammation is conditioned by the pH of the exudate. Subsequent studies revealed that the mechanism of local acidosis is often referable to a disturbance in the local intermediary carbohydrate metabolism and in the depletion of the alkali reserve (Menkin and Warner, 1937). When the lymphatic circulation and ultimately the vascular channels are obstructed, as is apt to occur in acutely inflamed tissue, the injured area becomes shunted off from the rest of the organism. It develops its own pH, its own fluid exchange, and its own metabolism. With such impairment in the local circulation, a state of relative anoxemia tends to develop. Perhaps an anaerobic glycolytic type of respiration may result. The plausibility of this view is supported by finding an enhanced glycolysis and a depletion in the alkali reserve. The lactic acid level of the exudate rises with a corresponding drop in sugar level. The observations thus indicate that the cytologic picture seems to be conditioned by the pH which, in turn, is frequently referable to disturbance in the local carbohydrate metabolism. The possible implications of these findings in regard to a clearer understanding of the histologic manifestations of various lesions are obvious. It is conceivable that the relative absence of leukocytic migration in the late stages of inflammation may be referable to a local increased concentration of lactic acid. This acid is, as has been pointed out above, negatively chemotactic. Its formation in appreciable amounts may thus counteract the migration of white cells induced by the liberation of leukotaxine (Menkin, 1938 b).

In the foregoing discussion an attempt has been made to trace the salient sequences involved in the development of an acute inflammatory reaction. The interdependence of the various manifestations concerned in the disturbance of local fluid exchange, increased capillary permeability, and leukocytic migration produce a complex mechanism which tends to localize and ultimately dispose of the irritant. The immunological aspects of this concept of inflammation will now be considered in detail.

II. *Inflammation in Relation to Immunity.* In the last few years evidence has been brought forward to show that the inflammatory reaction plays a definite rôle in localizing the irritating factor, or at least limiting its dissemination, whether it be a bacterial or a chemical irritant. This capacity, which may appropriately be termed *fixation*, contributes a further significance to inflammation in relation to immunity. By delaying the dissemination of obnoxious substances into the circulating blood stream, the inflammatory reaction protects the organ-

ism as a whole at the expense of local injury. This view of inflammation has gained considerable ground in the last few years. Analyses have demonstrated that fixation at the site of inflammation is primarily due to mechanical obstruction caused by a network of fibrin and to occlusion of lymphatic vessels (Menkin, 1931 a, c, and e). Studies on the time relationship have shown that with powerful irritants fixation is the initial phase in the development of a severe inflammatory reaction, and that it precedes even migration of leukocytes. In view of the available evidence one can ascribe a definite function to the early formation of lymphatic blockade at the site of inflammation. *Fixation* plays a definite rôle in the relation of inflammation to immunity. It may be considered in the same light as the migration of leukocytes and the stage of fibrous repair that follow.

a. *Local fixation of microörganisms and of inert substances at the site of an acute inflammation.* The dissemination of foreign substances from the site of injection into the lymphatics and into the blood stream has been studied by numerous investigators. Muscatello (1895) showed that carmine and various other inert particles, when injected into the peritoneal cavity, reached the anterior mediastinal lymph nodes very rapidly. Noetzel (1906) injected bacteria (*Bacillus pyocyaneus*) into the knee joints of rabbits; from five to ten minutes later he was able to demonstrate the presence of the organisms in the inguinal, crural, and lumbar lymph glands. Buxton (1907) found that typhoid bacilli, within a few minutes after their injection into the peritoneal cavity, appeared in great numbers in the blood stream. Wells and Johnstone (1907) showed that the absorption of bacteria from the peritoneal cavity takes place through lymphatic vessels. New and similar observations concerning normal lymphatic absorption have been reviewed in great detail by Drinker and Field (1933).

Does the inflammatory reaction retard the dissemination of microorganisms from the site of inflammation? The studies of Issayeff (1894) are interesting in this connection. He showed that the peritonitis induced by a variety of sterile irritants, such as foreign blood serum, bouillon or normal salt solution, temporarily increased resistance to subsequent intraperitoneal injection of bacteria. Pawlowsky (1909) repeated the observations of Noetzel and demonstrated the presence of staphylococci in the blood and organs of guinea pigs from twenty-four to forty-eight hours after inoculation of the knee joint. If before inoculation an acute inflammation of the knee joint had been produced

by the injection of a sterile irritant, such as turpentine, alcohol or quinine solution, the dissemination of the microörganisms was either inhibited or wholly prevented.

Rivers and Tillett (1925) confirmed these studies by finding that beef broth injected into the skin of rabbits protected against streptococci inoculated twenty-four hours later. No retardation was observed when the microörganisms were inoculated simultaneously with the beef broth. Mallory and Marble (1925) concluded that the local resistance produced by filtrates of staphylococci was due to local inflammation and was therefore not specific. It is to be recalled that Besredka (1925) advocated the therapeutic use of such filtrates. Favilli and McClean (1937) have also recently arrived at the conclusion that local immunity, as manifested in the skin, is due to a decrease in tissue permeability associated with the earliest phases of the inflammatory reaction. These investigators are unable to accept Besredka's view (1919) that local immunity is primarily a specific immune response. Gay believes that the phenomenon is referable to an enhanced non-specific resistance caused by the presence of macrophages (1935). Stuppy, Cannon, and Falk (1931) showed that insufflation with pneumococci induced a pulmonary immunity. These workers are of the opinion that, perhaps with the aid of humoral agencies, the induced tissue immunity is predominantly cellular in type. Opie (1929) showed that acute inflammation of the peritoneal cavity caused by aleuronat retarded the rush of injected hemolytic streptococci from the peritoneal cavity into the circulating blood and after twenty-four hours completely prevented it. By a culture method Menkin (1931 d) demonstrated that *B. prodigiosus* injected into an acutely inflamed area was fixed *in situ* and failed to disseminate into the tributary lymphatics.

As briefly stated above, some authors believe that in inflammation the large mononuclear phagocytes are responsible for protection. Nakahara (1925) injected olive oil into the peritoneal cavity and showed that after forty-eight hours an exudate was formed containing from 65 to 85 per cent of macrophages. At this stage the resistance of mice to Bacillus coli introduced into the peritoneal cavity was definitely increased. Gay and Morrison (1923) have studied the changes in resistance to intrapleural inoculation of streptococci after a sterile inflammation had been previously caused by various irritants. They contend that the increased resistance to the pathogenic microörganism results from the accumulation of clasmatocytes. This interpretation has been questioned by Opie (1929) who maintains that we have as yet no data that

will determine the rôle of polymorphonuclear leukocytes, mononuclear cells, serum, or indeed increased permeability of blood vessels in overcoming the injurious effects of sterile irritants or of bacteria. Gay and Clark (1930) in answer to these criticisms have produced some evidence showing that perhaps the polymorphonuclear leukocytes are relatively inactive in increasing the resistance of their animals. It is true that the increased resistance to virulent streptococci brought about by previously "preparing" a cavity with a sterile irritant is effective only when the inflammatory reaction has been going on in the cavity for 48 to 72 hours. Local fixation of substances at the site of inflammation, on the other hand, may be demonstrated within a relatively shorter time after the introduction of necrotizing inflammatory irritants (Menkin, 1929). With mild irritants, however, such as hemolytic streptococci, fixation may be delayed for as long as two days (Menkin, 1933 c). The increased resistance to intrapleural inoculation of streptococci found by Gay and his collaborators is possibly the resultant of several factors. It is difficult, on the basis of available evidence, to refer enhanced resistance to a single factor. Menkin has shown for instance that lymphatic blockade is a significant factor in evaluating resistance (1936 e) but, in the process of local disposal of an infecting pathogen, there are doubtless other elements concerned. These may include the cellular constituents of an exudate, the type and quantity of cells, as well as the rapidity of their appearance at the site of inflammation.

Freund (1931) studied the capacity of young rabbits to fix bacteria at the site of inoculation. This investigator observed that, whereas pneumococci induce a severe inflammation at the site of inoculation in adult rabbits, young animals, on the other hand, fail to develop an appreciable local reaction. Concomitantly he found that death occurs in relatively few adult rabbits, but that young rabbits succumb with pronounced bacteremia.

Menkin likewise studied the behavior of various foreign inert substances injected into an inflamed area by direct observation of the tributary lymphatic nodes and vessels draining the area. Trypan blue, ferric chloride, colloidal iron, graphite particles, and a foreign protein were found to be readily retained at the site of an acute inflammation (1929, 1930 a, b, 1931 d). Fixation of a dye could be demonstrated within thirty minutes following the injection of a strong irritant (e.g., aleuronat). Okuneff observed a delayed absorption of dye from the peritoneal cavity if the latter had previously been prepared by injection

with animal charcoal, casein, or gelatine (1924). The retarded passage of dye into the circulation was referred by this investigator to an adsorption phenomenon. The anatomical location of the inflamed area may be an additional factor in determining the intensity of fixation. For instance, Menkin (1930 b) observed that a foreign protein injected into a cutaneous inflammatory area was held *in situ* for a longer period than when injected into an inflamed peritoneal cavity. Miller recently confirmed the foregoing observations on the fixation of both trypan blue and foreign proteins (1938). In addition she noted the rapid absorption of diffusible substances from the site of inflammation provided the reaction had not progressed to abscess formation or necrosis. It is obvious, however, that an acute inflammation may develop to a suppurative state which involves some degree of necrosis or abscess formation. Substances that are either soluble or highly diffusible are readily retained only in areas of severe damage (Menkin, 1933c). The statement, however, that an intense degree of injury involving some necrosis is not an integral part of inflammation seems to be arbitrary and therefore unwarranted.

b. *The rôle of lymphatics in minor injury.* Employing intralymphatic injections of vital stains in mice, Hudack and McMaster (1931) pointed out that the least tissue injury induces an increase in lymphatic permeability. Furthermore, they showed that lymph readily escaped into areas of minor inflammation, such as were brought about by an arc light or by application of xylol. They concluded at the time that perhaps the lymph drainage in an inflamed area is impeded by an increased lymphatic permeability. This fact may perhaps account in part for the development of inflammatory edema.

In subsequent studies (1932) on human skin these investigators pointed out that even the slightest abrasion of the skin allows the passage of material into skin lymphatics. Material introduced by mere scratching, puncturing, or by intradermal injection immediately enters these vessels. They found that lymphatic capillaries in regions injured by heating, ultra-violet light, or by intradermal injection of bacterial toxins are definitely more permeable than under ordinary circumstances. Diffusible dyes injected into such areas escape with extreme rapidity in striking contrast to their behavior when placed in contact with normal cutaneous regions. In brief, they found that if the inflammatory process has not progressed to a purulent or to a necrotic stage there is a more rapid turnover of such material from the inflamed area.

They also studied the reaction of lymphatic capillaries in the ears of

mice immediately after minor injuries, such as are produced by incisions or mild burns (1934). Whereas small blood vessels tend to close after an incision, the lymphatics remain open for as long as forty-eight hours. Consequently materials introduced into such wounds pass directly into lymphatics through their gaping ends. This may explain the dissemination from cutaneous abrasions of infectious agents through lymphatics. As in the case of vascular capillaries, the lymphatic wall about an area of injury is rendered abnormally permeable. It is quite possible that this increased permeability is likewise referable to the liberation of leukotaxine (Menkin, 1938 a, b). Twenty-four to forty-eight hours later McMaster and Hudack found lymphatics less permeable than usual. Probably by that time the inflammatory reaction had reached a sufficient degree of intensity to favor plugging of lymphatics (Menkin, 1931 a, 1933 c, 1935 a). It is conceivable that the observations of Falchetti and Carlinfanti (1933) on the enhanced development of anaphylactic shock, when a homologous antigen is injected into an inflamed area induced by BCG, can be explained by the more rapid diffusion of the foreign protein from a site of relatively mild injury.

The observations of Hudack and McMaster are of great significance, for they clearly demonstrate that, in the initial stage of an acute inflammation, the lymphatic vessels undergo changes similar to those encountered in capillaries, namely, an increase in permeability. With a marked initial exudation from vascular structures it is therefore not surprising to note a parallel increase in lymph flow and pressure (Lassar, O. (see Cohnheim, 1889), Field, Drinker, and White, 1932). This would also hold true in mild types of inflammatory processes. When, however, the intensity of local injury or necrosis reaches a certain critical level lymphatic blockade ensues, as evidenced by the inability of foreign material or bacteria to disseminate readily from the site of acute inflammation (Menkin, 1931 a, 1933 c, 1935 b). In brief, the work of Hudack and McMaster on the rapid turnover of dyes from areas of minor injury is not in the least at variance with the observations of other workers on the local fixation of microörganisms in regions of severe inflammation. This fact was fully recognized by Hudack and McMaster (1933). The intensity of local injury determines the patency of the tributary lymphatic vessels.

c. *The accumulation and localization of foreign substances or bacteria in inflamed areas.* It has been known for some time that injury to tissue may determine the localization in the damaged tissue of bacteria or of ultrafilterable organisms present in the circulating blood. Calmette

and Guerin (1901) found that after the back of a rabbit had been shaved, intravenous injection of the virus of vaccinia resulted in a localization of the virus in the epilated area. Kettle (1924) showed that tubercle bacilli injected intravenously into mice or rabbits tended to settle in localized subcutaneous lesions caused by various agents that produce increased vascularity and necrosis of tissue. Falchetti (1933), on the other hand, rarely observed the localization of virulent tubercle bacilli in various subcutaneous lesions of guinea pigs. Chesney, Turner, and Halley (1928) found that when rabbits with wounds in their backs were inoculated intratesticularly or intravenously with *Spirochaeta pallida*, syphilitic lesions invariably developed in the wounds. Findlay (1928) believed that histamine-like substances, liberated at the point of injury, were the specific agents which by their action on the walls of the capillaries caused organisms present in the blood stream to localize at the site of injury. Sager and Mickel (1929) demonstrated that subcutaneous injection of silver nitrate into rabbits caused sterile abscesses. After intravenous injection of green streptococci, these organisms were recovered from the abscesses in some of the rabbits. They concluded that bacteria may become localized in places of lowered resistance. Quednau (1929) pointed out that bacteria, especially pneumococci, in the blood stream tended to localize readily in areas of cerebral softening. Burrows (1932) in a detailed monograph has adequately reviewed his observations, as well as those of numerous investigators, on the localization in inflamed areas of bacteria, viruses, dyes, proteins, and inorganic particles.

Reference has already been made to the retention at the site of an acutely inflamed area of various inert substances (e.g. dyes, iron, graphite, foreign proteins) as well as of bacteria (Menkin, 1929, 1930 a, b, 1931 d). Intravenous injection of these materials is accompanied by their accumulation and concentration in inflamed areas owing to their inability to escape readily through draining lymphatics (Menkin, 1929, 1937 b).

It is conceivable in view of these studies that the character or course of the inflammatory reaction may be altered by the accumulation from the blood stream and the subsequent fixation of various materials in an area of injury. In this connection, it has been demonstrated that repeated intravenous injections of ferric chloride into tuberculous rabbits are followed by deposition of the metal in the tubercles (Menkin, 1930 c; Menkin and Menkin, 1931). Concomitantly with this accumulation the progress of the experimental disease is retarded, as indicated

by an appreciable increase in the period of survival and by a difference in the extent of lesions in the lungs of control and experimental rabbits (Menkin, 1932 a, 1933 b, 1934 b, 1936 b). These earlier studies have recently been further substantiated by observations on the number of viable bacilli recovered after culturing some of the involved organs of both control and experimental rabbits (Menkin and Kadish, 1938). These studies, carried on for a number of years, represent the results of several independent series of experiments. The clinical implications are obvious, especially since the innocuousness of this iron salt in man has recently been pointed out (Menkin, 1937 c). It is also conceivable that one might find practical uses for a substance which by accumulating from the blood stream would enhance the roentgen appearance of various inflammatory lesions.

The accumulation and fixation of bacteria in an area of inflammation offers a reasonable explanation for the frequent localization of bacteria from the blood stream in a so-called *locus minoris resistantiae* following preliminary trauma or other tissue injury (Menkin, 1931 d; Burrows, 1932).

Since antibodies are regarded as being closely associated with globulins, the demonstration of an increased concentration and of a retention in inflamed tissue of foreign proteins from the blood stream would obviously be significant in connection with the rôle of antibodies in specific infectious processes. A series of experiments revealed that horse serum injected into the circulation accumulates at the site of an acute inflammation from which it fails to drain away readily (Menkin, 1930 b). The fixation of this foreign protein was found to be distinctly more pronounced in inflamed cutaneous tissue than in a correspondingly irritated peritoneal cavity. These observations formed the basis for the probable explanation of the local anaphylactic phenomenon described several years ago in the ear of the rabbit by Auer (1920). In their studies on local hypersensitiveness in the rabbit eye, Seegal and Seegal were able to make observations substantially supporting the previous findings on the accumulation of proteins in an inflamed area (1933). Recently Fox has further confirmed this view by showing that antibodies in the circulation tend to localize in areas of inflammation (1936).

Hunnicutt and Sutliff (1932) studied the penetration from the circulation of antipneumococcic horse serum into the consolidated lung of three cases of lobar pneumonia. These investigators failed to find at post-mortem a greater accumulation of horse serum in the consolidated

than in the normal lung tissue. The early work of Kline and Winternitz (1915) indicated that in the pneumonic lung the circulation is markedly impaired owing to the wide distribution of capillary fibrin thrombi. They pointed out at the time that this impairment of circulation seriously interferes with the possibility of intravenous therapy in pneumonia. These studies and those of Hanger (1930) may in part explain the outcome of Hunnicutt and Sutliff's observations in their few moribund pneumonic patients.

The studies on the accumulation and fixation of foreign proteins in inflamed tissue suggest perhaps a possible explanation for the phenomenon of the focal reaction in tuberculosis. When tuberculin is introduced into the blood stream of an animal with a tuberculous lesion, an intense inflammatory reaction may develop in the lesion. The mechanism of this focal reaction in tuberculosis is not understood. It is conceivable, in view of the experiments with horse serum, that, in a like manner, tuberculo-protein may accumulate from the blood stream in the inflamed tuberculous lesion and, by its presence there, induce an enhanced local inflammatory reaction (Menkin, 1930 b).

Sanarelli (1924) described the reaction which occurs when a preliminary intravenous injection of cholera vibrio is followed 24 hours later by an intravenous injection of a filtrate of colon bacilli. The animals (guinea pig or rabbit) succumb revealing at post-mortem examination an acute hemorrhagic inflammation of the gastro-intestinal tract, extreme congestion of the visceral organs, and an acute inflammation of the kidneys. Shwartzman (1928) described a similar cutaneous phenomenon with bacterial filtrates. This investigator injected the filtrate of B. typhosus into the skin of a rabbit. Twenty-four hours later when the same filtrate or a filtrate of a non-related organism, such as the meningococcus, is introduced into the circulating blood, hemorrhagic necrosis frequently appears at the site of the cutaneous injection. Hanger (1928) obtained essentially similar results with filtrates of B. lepisepticum. Menkin (1931 c, e), on the basis of his studies on the localization from the blood stream of foreign proteins at the site of an acute inflammation, suggested that the intense cutaneous reaction following the intravenous injection of a bacterial filtrate may be in part the result of a concentration of this substance in an area of the skin already inflamed. The reaction does not have to take place with all bacterial filtrates or with all inflammatory irritants. The extent of induced local vascular thrombosis, the degree of permeability of the capillaries, and the optimum of the synergistic action of two irritating

substances on one another when administered by two different routes may modify the morphological appearance of the final reaction. Such an interpretation of the phenomenon as an enhanced non-specific inflammatory reaction has been in large measure supported by the studies of Debonera, Tzortzakis, and Falchetti (1932), Freund and Smith (1934), Sickles (1931), Karsner and Moritz (1934), Freund (1934) and more recently by Moritz (1937). Bordet (1936) classifies the Sanarelli-Shwartzman reaction in the category of non-specific allergic phenomena. This investigator points out that as a preliminary requirement the prepared skin area must manifest some slight edema in order to induce the phenomenon. This can be attained by a variety of means besides inoculation of bacterial filtrates. For instance, two successive cutaneous inoculations, twenty-four hours apart, of milk in one skin area followed several hours later by an intravenous injection of a filtrate of B. coli will induce a characteristic hemorrhagic reaction. Tuberculin injected into the skin of a tuberculous rabbit may induce a slight cutaneous thickening. On the next day the intravenous injection of B. coli filtrate is followed several hours later by a typical Shwartzman reaction in the tuberculin treated area. Gerber (1936) on the basis of histological studies states that "no doubt, the inflammation following skin preparation may, in the manner of any non-specific inflammation, attract to the local site the intravenously administered filtrate. Yet this does not explain the entire phenomenon." Shwartzman and Gerber therefore take exception to Menkin's point of view. They believe that "the preparatory factor induces a state of vulnerability of the tissue which is not in the nature of a mere trauma, increased permeability of capillaries or inflammation, but which is probably due to some functional disturbance in the cells which requires a short incubation period for its appearance and which disappears rapidly." This opinion is not as yet supported by any decisive experimental facts and is therefore at least somewhat conjectural. Menkin (1935 a) pointed out that reliance on histology alone as evidence for capillary damage in the prepared cutaneous site is fraught with difficulties. The absence of gross or microscopic evidence of inflammation in the prepared skin area by the use of diluted filtrate (Shwartzman, 1935) does not necessarily indicate that the capillary wall is normal. The increase in the permeability of capillaries in inflammation may occur extremely early and may, in many instances, produce little, if any, change that is detectable by gross or even microscopic examination (Menkin, 1933 c, 1935 a). An increase in the functional filtration through the capillary endothelium, as indi-

cated not necessarily by morphological evidence, but rather by the ability of foreign materials or bacteria from the blood stream to accumulate in the prepared area, would perhaps explain the Sanarelli-Shwartzman phenomenon as a peculiar manifestation of a non-specific inflammatory reaction induced by the superimposition of an irritant that concentrates from the circulating blood in a previously inflamed area. In this connection it is, however, to be noted that Bordet (1936) was unable to detect any accumulation of trypan blue in the prepared skin area when he substituted an intravenous injection of the dye for the filtrate on the day following the cutaneous inoculation. On the other hand Moritz (1937) reported that when a local state of hypersusceptibility was elicited by the subcutaneous injection of *B. aertrycke* filtrate, the subsequent intravenous injection of living *B. aertrycke* led at the prepared site to the development of a hemorrhagic necrosing inflammation in which the bacteria localized.

d. *Fixation of bacteria and of foreign proteins by the specific inflammatory reaction.* 1. *Allergy and immunity in tuberculosis.* Koch (1891) discovered an important principle in regard to the mechanism of immunity in tuberculosis when he described the reaction that takes place when a tuberculous guinea pig is reinoculated with either living or dead tubercle bacilli. This reaction to reinfection is termed the Koch phenomenon. In brief, Koch found that within ten to fourteen days after a healthy guinea pig is inoculated with a pure culture of tubercle bacilli, a hard nodule appears at the site of inoculation; this nodule ultimately breaks down and ulcerates. At the same time the tributary lymph node becomes enlarged and caseous. An entirely different picture develops when a tuberculous guinea pig is reinoculated four to six weeks following the primary infection. An acute inflammatory reaction occurs within a few hours at the point of reinjection without involving to any significant extent the satellite lymph nodes. von Pirquet (1906) proposed the term "allergy" for the altered response of tuberculous tissue to reinfection. The tuberculin reaction, which may be regarded as a manifestation of the Koch phenomenon induced by tuberculo-protein, is histologically an acute inflammation. During the first forty-eight hours there is an abundant accumulation of serum and polymorphonuclear leukocytes which are later displaced by an infiltration of mononuclear phagocytic cells (Opie, 1929). These observations, as will be pointed out, are at variance with the findings of Dienes and Mallory (1932).

Krause (1916) expressed the belief that specific immunity to reinfec-

tion in tuberculosis may be a function of allergy in so far as the inflammation accompanying the latter prevents the spread of reinfecting bacilli. The earlier work of Issayeff and of Pawlowsky cited by Opie (1910) suggested this view to Krause. Krause and Willis (1924) demonstrated experimentally the extent of the retardation in the infected animals. Willis (1925) studied the dissemination of tubercle bacilli from the site of inoculation and observed that in reinfected guinea pigs the spread of tubercle bacilli from the site of inoculation was retarded, whereas in normal animals the organisms passed readily to the regional lymph nodes. Both Krause and Willis emphasized the mechanical barrier set up by the inflammatory reaction against the outward movement of the deposited bacilli. In brief, their view was expressed in the following words: "We believe, therefore, that specific tuberculo-immunity occurs through a fixation of germs that results from the operation of the allergic reaction. An almost immediate inflammatory outpouring hems in the bacilli more or less effectively and thus delays or prevents their spread, which is so facile and rapid in the non-tuberculous, non-allergic animal" (Krause, 1925).

This view, which singled out the Koch phenomenon as practically the sole mechanism involved in the mechanism of immunity in tuberculosis, was severely criticized by Rich and McCordock (1929). They pointed out at that time that although it had been assumed that inflammation *per se* can limit the immediate spread of microörganisms, there was not the slightest proof of this "And until we have more precise information on the question it must be remembered that the inoculation results in the experiments of Krause and Willis might be interpreted on the basis of the well-established fact that there occurs a much greater destruction of tubercle bacilli in immune animals than in normal ones." As pointed out by the writer (1931 c), an interpretation of this sort is difficult to accept in view of the facts obtained. Willis (1925) demonstrated that the regional lymph nodes in immune animals were infected at the end of two weeks after cutaneous inoculation. If the apparent retardation of bacilli at the site of inoculation was primarily due to their destruction *in situ*, their appearance after a latent period of two weeks in the regional lymph nodes would be difficult to explain. Furthermore, Menkin (1931 d) showed that an acute inflammatory reaction caused by a non-specific irritant prevented for at least several hours the dissemination of *B. prodigiosus* to the tributary lymph nodes. A distinctly larger number of microörganisms were recovered from the site of inflammation than from the corresponding normal control area. These experiments

proved that an acute inflammation *per se* retards the dissemination of bacteria into the regional lymphatics. Rich and McKee (1934), in a discussion of some of these results, readily agreed that an acute inflammation is of assistance in preventing the dissemination of bacteria. However, they correctly pointed out that this phenomenon is only exhibited when the inflammatory reaction has attained sufficient intensity to induce a walling-off of the affected area. In other words, on the basis of Rich's view, the observations of Issayeff (1894) and of many other investigators, including the writer, fail to explain the immediate immobilization which takes place upon reinoculation of an immune animal. The studies of Menkin (1933 c), however, demonstrated that the rapidity with which an area of acute inflammation becomes walled off so that foreign substances fail to spread readily from it is a function of the irritant. He observed that strong irritants, as *staphylococcus aureus* or aleuronat, produce a prompt fixation which may occur as early as thirty minutes to one hour after the introduction of the irritant (Menkin, 1935 a). Mild irritants, on the other hand, produce a delayed reaction of fixation allowing relatively free penetration of the irritant into the circulation for a considerable interval of time. Hemolytic streptococci were shown to exemplify this type of irritant. This question of the immediate spread of bacteria at the onset of an acute inflammatory reaction was again discussed by Menkin (1936 c). He pointed out at the time that doubtless some, but not *all*, of the microörganisms disseminate at once from the site of any tissue inoculation. In a non-specific inflammatory reaction there is nothing to restrict the dissemination of some of the bacteria immediately after their inoculation and prior to the development of lymphatic blockade. The studies of Menkin (1929), of Hudack and McMaster (1932), and of Lurie (1936) indicated that there seems to be an increase or at least an unimpeded lymphatic drainage in the very initial stage of an acute inflammation. At the same time it is clear that the rapidity and intensity with which the inflammatory reaction develops will determine the ultimate number of microörganisms capable of penetrating to the circulating blood stream. There is, therefore, little doubt that without the developing allergic inflammation in tuberculosis the spread of the reinfecting bacilli would be unobstructed. The number of bacilli disseminating in the earlier stage of reinfection prior to the development of a sufficiently intense allergic inflammation are probably few, and therefore can be disposed of adequately in the tributary lymphatic nodes, i.e., provided their virulence is not too great.

Rothschild, Friedenwald, and Bernstein (1934) reported that tuberculous guinea pigs could be completely desensitized by a prolonged course of subcutaneous injection of Koch's Old Tuberculin. They were able to show that this complete desensitization fails to deprive the treated animals of any aspect of their immunity as measured by the course and severity of local lesions, by the spread of their visceral lesions, and by the mortality from the disease of infected animals. They claim that in these respects the desensitized animal manifests a resistance equal to or superior to that of the non-desensitized control. Furthermore, they concluded that: "In so far as the inhibition or spread of lesions from the site of infection to the viscera may be used as evidence of the local fixation of bacilli, the desensitized, non-allergic immunes were able to resist the spread of infection as successfully as the allergic immunes." On the basis of such observations, which are in agreement with the findings of several other investigators (Branch and Cuff, 1930; Boquet, 1933; Rich, Chesney, and Turner, 1933; Clawson, 1933), Rich has come to the conclusion that the elimination of hypersensitiveness fails to reduce the degree of immunity. He believes that the exaggerated inflammation manifested in reinfected hypersensitive animals is unnecessary (1929, 1936). In this connection the studies of Long and his collaborators (1933) indicated that the degree of natural immunity in various animals seems to be inversely proportional to the amount of inflammation developed by corneal infection with tubercle bacilli. In brief, Rich is thoroughly convinced that acquired immunity is in no way dependent on the development of allergic inflammation.

In discussing the results of Rothschild and his collaborators, Topley and Wilson (1936) point out that there may be a fallacy in the argument of these investigators. The assumption in their experiments presupposes that the only effect produced is a desensitization of the animal. The possible immunizing effect of repeated administration of massive doses of tuberculin is, according to Topley and Wilson, altogether ignored. It is conceivable that the experimental elimination of hypersensitiveness does not preclude the possible importance of allergic manifestations when all the other factors concerned in the mechanism of immunity are evaluated. At present we are unfortunately still ignorant as to whether or not other compensatory factors are brought into play during the process of desensitization to promote resistance.

In general it may be said that the extensive studies of Rich and of his collaborators have thrown some doubt on the widely accepted view that allergy is the essential mechanism of immunity in tuberculosis (Krause

and Willis, 1924; Zinsser, H., Ward, H. K., and Jennings, F. B., Jr., 1925). The basic observations of Lurie have indicated that immunity in tuberculosis is "a function of the increased capacity of the mononuclear phagocytes to digest tubercle bacilli" (1933, 1936). His earlier work has suggested the presence of an additional differential local organ immunity. In the spleen, liver, and bone marrow of an infected rabbit tubercle bacilli are destroyed more rapidly than in the lungs and kidneys (Lurie, 1928). In the allergic animal these same differences are essentially maintained, producing merely an enhanced destruction of the reinfecting bacilli (Lurie, 1929). The studies of Lurie support the view that besides allergy there are other factors involved in the mechanism of immunity in tuberculosis. Genetic factors and racial differences in the human disease are probably additional important elements (Opie, McPhedran, and Putnam, 1936, Lurie, 1937). In brief, the view that resistance may be at times in large measure independent of allergy and thus be referable to other subtle immunologic factors is not questioned (Menkin, 1931 c).

On the other hand, the conclusions drawn by Rothschild and his associates (1934) have, as mentioned above, been questioned (Topley and Wilson, 1936). In this connection it is to be remembered that desensitization to cutaneous inoculation of tuberculo-protein is no proof that the animal is completely desensitized. Freund (1929) demonstrated that the intracutaneous tuberculin test is negative or only very slightly positive in young guinea pigs; but at the same time the animals are as sensitive to the toxic action of old tuberculin introduced into the peritoneal cavity as are adult tuberculous guinea pigs. This investigator concluded that systemic hypersensitiveness is actively developed in young guinea pigs at a time when the dermal reaction is very slight or negative. These observations merely suggest the necessity of considerable caution in inferring complete desensitization by the absence or elimination of cutaneous reactivity. Furthermore, Woodruff and Willis (1937) recently repeated the experiments of Rothschild. These investigators allowed the animals to die of their disease. Under these circumstances they found, contrary to the observations of Rothschild and his collaborators, that the desensitized guinea pigs survived a shorter interval; these animals revealed more pulmonary involvement; and finally the various tissues studied contained more tubercle bacilli, as demonstrated by Lurie's culture technique (1928), than did control guinea pigs that had not been desensitized.

In conclusion, the fundamental issue is this: When an allergic in-

flammation develops as a result of reinfection, does the inflammatory reaction play a definite rôle in the mechanism of immunity by retarding the dissemination of bacteria? The various types of evidence already cited as well as others to be discussed later, confirm the viewpoint that an acute inflammation, either of specific origin or caused by a non-specific irritant, definitely delays the spread of injurious substances from the site of inflammation. The principle of the Koch phenomenon applied to human tuberculosis is probably still the best available explanation to distinguish adult from childhood tuberculosis. The various studies of Küss, Ghon, and Opie indicate, in the words of the latter (1936), that: "The peculiar characteristics of adult or reinfection type of pulmonary tuberculosis are referable to allergy induced by a first infection." The available facts therefore strongly suggest that the allergic inflammation is quite likely one factor among several others which is involved in the manifestation of immunity in tuberculosis. Its importance lies in the tendency of the acute inflammation of hypersensitiveness to restrain the free penetration of reinfecting bacilli. Unfortunately at present there seems to be no available method of evaluating in a quantitative manner the relative significance of each of the various factors involved in the mechanism of immunity in tuberculosis.

2. *Fixation of pyogenic bacteria in immune animals.* Wadsworth (1904) injected virulent pneumococci into the trachea of normal animals and thereby caused a fatal bacteremia which in some cases was associated with bronchopneumonia. When immunized animals were treated in the same manner, there was a diffuse exudation comparable with lobar pneumonia, but no invasion of the blood stream. Cecil and Blake (1920) also found that the incidence of invasion of the blood stream was less in monkeys vaccinated against pneumonia induced by intratracheal inoculation than in nonvaccinated animals. Cannon and Pacheco (1930) described histologic studies of the skin and subcutaneous tissues of the abdominal walls of normal guinea pigs and of animals previously immunized by intracutaneous injections of a staphylococcal vaccine. All the animals were infected by the intracutaneous injection of a live virulent culture of *Staphylococcus aureus.* In the normal animals the staphylococci disseminated throughout the subcutaneous tissues as a cellulitis. In previously immunized animals, on the other hand, the staphylococci tended to remain localized near the site of inoculation. These investigators expressed the belief that the immunity secured was predominantly cellular, with the tissue macrophages playing the dominant part. Localization of the microörganisms was also rein-

forced to some extent by the action of agglutinating or of opsonizing antibodies. Angevine (1934, 1936) demonstrated in the skin of immune rabbits a retardation in the passage of avirulent and virulent streptococci from the site of their inoculation to the regional lymph nodes. He found, after immunization associated with sensitization, that virulent streptococci were more readily localized in the skin and destroyed by phagocytosis. With a relatively avirulent strain of hemolytic streptococci he observed in sensitized animals a marked local inflammation. The microörganisms disseminate from such an area to the tributary lymphatic nodes in appreciably smaller number.

Rich and McKee (1932, 1934) have come to the conclusion that local immobilization of bacteria (pneumococci, type I) in a reinfected immune animal is primarily due to the presence of agglutinating tissue antibodies which prevent the dissemination of the microörganisms. The localization is immediate and precedes the development of fixation by inflammation. According to Rich (1933), when the acute inflammatory reaction is finally established, it no doubt is of assistance in preventing the further spread of bacteria. The writer (1930 b), on the basis of his earlier studies on the concentration of proteins in an inflamed area, agrees essentially with this viewpoint. The question, however, is raised whether the presence of part of the tissue agglutinins in an immune animal is not the result of accumulating antibodies from the circulation. Inflammation, as has been pointed out (Menkin, 1933 c), may at certain initial stages reveal other manifestations besides leukocytic migration. The early increase in capillary permeability, although perhaps not detectable microscopically, is nevertheless as much a part of the inflammatory reaction as is the subsequent infiltration of leukocytes. Consequently the conspicuous concentration of antibodies in an area of reinfection does not necessarily imply a phenomenon independent of inflammation. The usual antibody content in a local area of an immune animal (Freund and Whitney, 1929) may thus be simply enhanced by a further accumulation of agglutinins through the damaged capillary wall. The prompt concentration of antibodies from the circulation in an area of specific inflammation thus reinforces the basic mechanism that tends to inhibit the spread of bacteria.

3. *Fixation of foreign proteins by the specific inflammatory reaction.* Extensive studies by Rössle (1914) and especially by Opie (1924 a, b) demonstrated that a homologous antigen injected into the skin of a sensitized animal is fixed at the site of injection. This fixation is accompanied by an acute inflammatory reaction (Arthus phenomenon).

When a small quantity of a foreign protein, such as horse serum or egg white, is injected into a normal rabbit, it is demonstrable by the precipitin reaction in the blood of that animal for a period of from seven to nine days. Opie showed that, with continued immunization, injected proteins exhibit a decreasing tendency to find their way into the blood. After a few immunizing injections they can be found in the blood serum in only small quantities and for but a short time. After the sixth or seventh injection strongly immunized animals reveal no trace of the proteins in the circulating blood. By excising and extracting with physiological saline the cutaneous site of injection in normal and immune rabbits Opie demonstrated that in the immunized animal fixation of the antigen at the site of injection prevents its dissemination throughout the body. Opie (1925) expressed the opinion that specific precipitation plays a part in this fixation. The precipitate formed by the contact of antigen and antibody has a strong attraction for polymorphonuclear leukocytes of the blood and, according to Opie, is doubtless digested and destroyed by these cells. In a later study Menkin (1930 b) demonstrated that a non-specific acute inflammatory reaction is capable of arresting a foreign protein at the site of injection. For this reason it is probable that the fixation of antigen in the Arthus phenomenon is largely due to the acute inflammatory reaction brought about by the contact of antigen and antibody in the tissues. Opie (1925) pointed out that it is unnecessary to assume that the tissues have undergone any essential alteration as the result of sensitization. "Acute inflammation in response to a specific irritant, namely, Arthus phenomenon, occurs when the antigen concerned and its antibody meet within the tissues." This conclusion was reached by reversing the usual procedure. A normal animal received 10 cc. of horse serum intravenously. On the following day 0.5 cc. of strong antihorse serum from an immunized rabbit was injected into the skin. Extensive inflammatory edema (Arthus phenomenon) occurred at the site of injection, whereas none followed the injection of normal rabbit serum. Opie, contrary to Doerr (1936), viewed the anaphylactic inflammation in which the antigen is fixed at the point of entry as an apparent paradox between susceptibility to injury and the resistance of immunity. With the anaphylactic inflammation the vital organs are protected at the expense of local injury. Nordmann has suggested as an additional explanation that there is a physiological adaptation of the arteries which is ultimately responsible for the necrosis occurring in the Arthus phenomenon (1931). It is doubtful whether this author's criticisms of Opie's in-

terpretation of the phenomenon invalidates in any way the observations and inferences drawn by Opie. The issue involved has, however, already been discussed at length and space prevents any further reference to the question (Menkin, 1931 c).

Dienes undertook a study of the mechanism of bacterial allergy (1927, 1932, 1936). He demonstrated that in the course of sensitization to a foreign protein there is a preanaphylactic period in the first few days of immunization during which a cutaneous reaction may be elicited. This early response manifests certain similarities to the tuberculin reaction. Its occurrence precedes the presence of circulating precipitin antibodies. Its histologic picture is characterized by an initial infiltration of mononuclear phagocytes in contrast to the local anaphylactic response in which the first cellular elements are primarily polymorphonuclear leukocytes. These histological findings are not fully in agreement with the observations of Opie (1929) and of Feldman and Fitch (1937). Laporte (1934), however, has essentially confirmed the findings of Dienes and Mallory. As pointed out by Laporte and more recently by Feldman and Fitch, the discrepancy in results may be referable to various factors such as the species of animal sensitized, the degree of hypersensitiveness, and the quantity of tuberculin utilized. At any rate Dienes expressed the opinion that in the early forms of protein sensitization, which seem to resemble the classic allergic manifestation (e.g., tuberculin reaction), the antibodies are probably in close association with cells. Curiously enough Dienes was able to demonstrate that a similar sensitization with egg white or horse serum performed in tuberculous guinea pigs intensifies the cutaneous reaction upon reinjection of the homologous antigen. It is conceivable that the presence of an established tuberculous infection retards the dissemination from the skin of a protein antigen during the course of immunization. This may induce in the skin an increase in the local concentration of antibodies. Such a state of affairs would perhaps give rise to a more intense cutaneous reaction upon reinoculation of the antigen. The recent work of Joyner and Sabin (1937) is of interest in this connection. These authors pointed out a significant retardation in the spread of vital dyes from the skin of tuberculous guinea pigs. The work of Rich (1936) is also suggestive. He found that intradermal reinjection of pneumococci 24 hours after primary skin inoculation with the same microörganism, but at a different site, induces in the rabbit the development of a circumscribed lesion. In unpublished experiments on the rabbit Menkin and Warner (1936) observed that the apparently normal skin of the abdomen

frequently displays an increased glycolysis when an acute cutaneous inflammation has been set up at some distance from the normal area. It is to be recalled that these workers brought forward evidence indicating that the severity of a lesion seems to be directly related to the intensity of glycolysis (1937). Biochemical observations of this sort suggest that perhaps a local cutaneous inflammatory reaction may be of significance in altering the normal metabolism of a considerable area of what is otherwise considered uninvolved skin. This possibility may have to be taken into account when studying absorption of material from the site of skin inoculation.

e. *Mechanism of fixation by the acute inflammatory reaction.* The mechanism responsible for the fixation of various substances at the site of an acute inflammation seems to rest primarily on the establishment of an effective lymphatic blockade (Menkin, 1931 a, 1932 b, 1937 b). This is brought about by two factors, the presence of each of which may vary in extent.

The mechanical barrier which circumscribes an area of acute inflammation consists of coagulated plasma in tissue distended with edema, and, if the reaction becomes sufficiently intense, the afferent lymphatic vessels become occluded by thrombi. It is quite likely that when the inflammatory reaction is fully developed other elements may participate in intensifying the retention of material in the injured area (Menkin, 1931 a, 1937 b). In this connection leukocytes may, in the later phases of the inflammatory reaction, play some definite rôle through their phagocytizing properties. Menkin (1930 b) has previously demonstrated the prompt accumulation of foreign proteins from the circulation in inflamed foci. Fox (1936) has also recently obtained evidence suggesting that antibodies tend to localize in such areas. In view of these facts, it is therefore reasonable to suppose that in an allergic or anaphylactic inflammation the mechanism of fixation is probably reinforced by the presence of specific antibodies. This view was foreshadowed by Opie (1924 a, b) in his investigations on the mechanism of the Arthus phenomenon, and it was later developed further by Rich and McKee (1934) in their interesting studies on the immune animal.

Opie (1936) believes that it is difficult to estimate the relative importance of leukocytes, antibodies, and of the inflammatory reaction itself in local fixation. Careful analyses, however, of the mechanism involved in regard to the element of time, or what may be termed the threshold of fixation, supports the view that lymphatic blockage constitutes the basic reaction but that, as often maintained, it may be rein-

forced by various factors (Menkin, 1931 a, 1932 b, 1933 c, 1937 b). Whereas the latter, in turn, may be quite significant, they are nevertheless considered secondary for the simple reason that they do not have to be present for the reaction of fixation to take place. As has often been pointed out by the writer, his observations indicate that with some powerful irritants, which cause considerable necrosis (for instance, aleuronat), fixation of foreign material may occur as early as thirty minutes after introduction of the irritant, at a time when there is as yet a scarcity of leukocytes in the extracapillary spaces. Furthermore, microscopic studies at this period fail to reveal any trace of such foreign material in phagocytes. Yet the foreign substance is prevented from reaching the regional lymph nodes. Conversely, in studies of various types of acute non-allergic inflammatory reactions, Menkin consistently failed to observe any sign of fixation of the tested material without some degree of occlusion of the afferent lymphatic vessels and generally the presence of a network of fibrin (1937 b). The lymphatic channels were either completely or in part blocked by thrombi. In tissue distended with edema the degree of fixation was usually well correlated with the extent of fibrin deposition, or of lymphatic thrombosis, or with both of these factors. For instance in some types of acute dermal inflammation, as induced in the rabbit with pneumococci type I, Menkin (1933 c) noted the scarcity of fibrin deposits in the involved spaces. However, the fixation of a vital dye in a cutaneous area inoculated with these microorganisms was invariably correlated with the finding of thrombi in the draining lymphatic vessels. On the other hand, in inflammatory reaction induced by a burn (e.g., with hot water) the retention of material was primarily correlated with the finding of fibrin in the extracapillary spaces rather than with the presence of conspicuous lymphatic thrombosis (Menkin, 1933 a). This investigator therefore concludes that the combination of both factors or the prevalence of either one seems to be a necessary requisite for fixation to occur at the site of an acutely inflamed area (1937 b).

The evidence for arriving at this conclusion has been described in detail elsewhere (Menkin, 1935 a) and therefore will merely be restated here in brief:

1. Morphological examination substantiates the view that fixation may well be referable to lymphatic blockade caused by fibrin and obstructing thrombi in lymphatic channels.

2. It was reasoned that, if mechanical obstruction plays an important rôle in fixation, then substances which are unable to disseminate from

an acutely inflamed area should, for the same reason, fail to enter it when injected at the periphery of the area. This is precisely what was found to occur when trypan blue or bacteria were injected at the periphery of an inflamed focus. Burrows has recently confirmed this experiment with India ink (1932). Studying the living frog under the microscope, the writer has followed the diffusion of a dye from its peripheral inoculation to the boundary of the inflamed area where its further progress was blocked by the presence of a thrombus occluding a lymphatic channel (1931b).

3. A third type of evidence which reinforces the hypothesis was obtained by taking advantage of the fact that concentrated urea dissolves fibrin. When this solvent was injected immediately after the irritant, obstructing thrombi failed to form in the tributary lymphatics, and fixation was inhibited. Urea had apparently no other manifest effect on the cytological character of the exudate as revealed by microscopic examination. These three distinct types of evidence render it quite likely that the basic mechanism of fixation in inflammation is primarily referable to an obstructing barrier in the form of thrombi in lymphatics and of coagulated plasma in edematous tissue. There is as yet no precise information on the question of the relation of increased capillary passage to the lymph flow from an inflamed area. Pending such data, the finding of increased lymph flow from a main lymphatic trunk draining a large area of tissue injured unequally by the inflammatory irritant only indicates, as would be expected, an absolute increase in lymph flow. In such an experiment one cannot preclude the possibility of a relative decrease in the lymph output when the total amount of plasma which has permeated through the capillaries is taken into consideration (Menkin, 1933 a). Inflammatory edema may in part be an index of the inability of a partially impaired lymphatic circulation to take care of the excessive passage of fluid from the circulating blood into acutely inflamed tissue. In this connection the interesting observations of Drinker and Field are suggestive and instructive (1933). These investigators have succeeded in developing a conspicuous local edema or elephantiasis in the leg of dogs following obstruction of the lymphatic trunks.

Burrows (1932) recently reviewed the various factors involved in the mechanism of inflammatory retention. According to this author these may be briefly stated to be as follows: *a*, increased osmotic pressure; *b*, stasis and clotting of lymph; *c*, phagocytosis; *d*, agglutination of bacteria *in vivo*, and *e*, the electrostatic conditions in the tissues as well as

the electrical charges on the particles which are retained in an area of acute inflammation. Schade and Menschel (1923) have found that in inflamed areas, especially those with suppuration, the accumulation of products of tissue disintegration may become so great that the osmotic pressure is raised to as high as eleven atmospheres, with a simultaneous marked increase in hydrogen ion concentration. They believe that inflammatory edema has primarily an osmotic origin. For this reason it seems reasonable enough to suppose that the increase in osmotic pressure at the site of inflammation may be a factor in influencing the flow of fluids and perhaps indirectly any contained substance. It is, however, doubtful whether this factor plays a significant rôle in fixation. In the first place determination of the osmotic pressure of exudates obtained in the *early* phase of an acute inflammatory reaction usually yields a value not markedly different from that of blood serum (Menkin and Warner, unpublished). Yet the fixation of various substances at such an early period in the development of an acute inflammation can be readily demonstrated. Furthermore, the fact that substances injected at the periphery of an acutely inflamed area fail to penetrate into it (Menkin, 1931 a) seems to invalidate the view that retention is primarily referable to an increase in osmotic pressure within the injured tissue. As pointed out previously, phagocytosis and agglutination *in vivo* are doubtless important secondary factors. Fixation, however, can be readily demonstrated in the absence of these factors (Menkin, 1937 b). When brought into play they are of considerable significance in reinforcing the basic mechanism of lymphatic blockade. The suggestion of differences in electric charges to account for fixation is an interesting view, which, however, seems as yet to have little experimental basis to substantiate it.

In brief, the evidence on hand which has received further support from the studies of Burrows (1932), of Clark and Clark (1937), and of Dennis and Berberian (1934), indicates that the basic mechanism of fixation in an acute non-specific inflammation seems to be primarily referable to the establishment of an effective lymphatic blockade (Menkin, 1931 a). Other elements, such as specific or non-specific precipitation, agglutination *in vivo*, size of particle or diffusibility, adsorption, or phagocytosis represent various secondary factors which, under special circumstances or at some phase of the inflammatory reaction, may be of great significance in reinforcing the primary and basic mechanism of fixation.

f. *Inflammation and bacterial invasiveness.* The foregoing observa-

tions on the mechanism of fixation have offered a means of studying a factor that may account in large part for the differences in the invasive ability of various pyogenic organisms.

The intensity and rapidity with which an inflammatory irritant is circumscribed in a tissue area varies with the type of irritant. The speed with which an irritant causes a region to be walled off by thrombosed lymphatics, or a fibrinous network, or by both, is necessarily an important index in determining its ability to disseminate ultimately into the circulating blood. This holds true if the irritant can readily drain through lymphatic vessels from the site of inoculation. That bacteria disseminate from the site of inoculation through lymphatics has frequently been demonstrated (Noetzel, 1906; Wells and Johnstone, 1907). It follows from this that, if bacteria are employed as inflammatory irritants, some information may be obtained concerning the invasive capacities of different microörganisms. This would depend on the type of inflammatory reaction which a given microörganism induces in the host. In other words, can the behavior of various pyogenic organisms in tissues be related, at least in part, to their respective abilities of inducing an early inflammatory reaction at the site of their inoculation which, by lymphatic blockade, would tend to prevent their dissemination to the tributary lymphatic nodes?

In observations described fully elsewhere (Menkin, 1933 c, 1935 a) it was definitely shown that the respective differences in the invasive behavior of *Staphylococcus aureus*, pneumococci, and hemolytic streptococci was in large part referable to differences in the rapidity and intensity of injury induced by these respective microörganisms. A culture of *Staphylococcus aureus* or of its soluble toxin[4] induces prompt lymphatic blockage, as indicated by histological examination of the site of inoculation, and also by the fact that within one hour after infection an injected indicator dye fails to reach the regional lymphatic nodes. In the case of hemolytic streptococci, however, about two days are required after their cutaneous inoculation before the local reaction is of sufficient intensity to induce lymphatic obstruction. These observations suggest a paradoxical interpretation. Staphylococci produce in general little systemic effect owing to their intense local injurious reaction which fixes them *in situ*. Streptococci produce marked generalized

[4] The effectiveness of the toxin of *Staphylococcus aureus* in inducing lymphatic blockade is destroyed by heating it to about 58°C. for one hour. The action of the toxin seems to be caused by the presence of a factor similar, if not identical, to leukocidin (Menkin, 1935a).

effects on the organism as a whole because of their comparatively mild local reaction. This allows them relatively free penetration to the essential organs.

Dennis, who, with Berberian, confirmed these observations on the mechanism of invasiveness (1934), has recently advanced the idea that hemolytic streptococci have the specific capacity of inhibiting the formation of a circumscribing fibrinous barrier (1936). This investigator's conclusions are perhaps open to some question inasmuch as in his experiments the possibility of reducing the activity of the irritant by mixing it *in vitro* with hemolytic streptococci is not precluded. The writer, in turn, has been unable to alter qualitatively the reaction of fixation by inoculating hemolytic streptococci into an area of tissue, either immediately following its injection with aleuronat, or at intervals of 20 to 30 minutes later (1937 b).

It is improbable that the interesting observations of Tillett and Garner on the fibrinolytic activity of hemolytic streptococci can offer an adequate explanation for the delayed fixation of trypan blue (1933). Their own findings definitely show that this lytic effect fails to take place with the plasma clot of the rabbit which was the animal used in the writer's own studies. It is more likely that in the case of human streptococcal infection the dissolution of fibrin, if it occurs *in vivo*, acts as an agent synergistic to a more fundamental factor which holds true for both human and rodent types of infection. Additional evidence has recently been obtained which indicates that the primary difference in the disseminating behavior of staphylococci and hemolytic streptococci is probably referable to varying degrees of local injury. Menkin and Warner have recently shown that the severity of an inflammatory reaction can often be graded by the extent of local disturbance in the intermediate carbohydrate metabolism (1937). Lesions induced by the *Staphylococcus aureus* manifest a considerably enhanced glycolysis in comparison to lesions of the same duration caused by hemolytic streptococci (1936 d). The average concentration of glucose was found to be 62.3 mgm. per 100 grams of tissue in cutaneous areas previously inoculated with *Staphylococcus aureus*. In skin areas of corresponding duration but injected with hemolytic streptococci, the concentration of sugar averaged 129.7 mgm. The level in normal skin was 149.9 mgm. These comparative figures indicate marked glycolytic activity in lesions induced by *Staphylococcus aureus*. This chemical difference furnishes additional evidence supporting the view that the respective invasive

behavior of these two microörganisms seems to bear an inverse relation to the intensity of their local lesions.

A number of authors, including Much (1908), Gratia (1920), and Gengou (1933) have shown that the *Staphylococcus aureus* and its exotoxin are capable of causing oxalated blood to clot. According to Gratia this is due to a thermostable substance which he calls *staphylocoagulase*. Menkin and Walston (1935) have succeeded in separating *staphylocoagulase* from the fraction that induces inflammatory fixation. This was attained by treating the filtrate of a culture of microörganisms with glacial acetic acid. They found that the "clotting principle" elaborated by staphylococci, when injected intracutaneously, fails to exert a sufficiently powerful local reaction to obstruct lymphatic drainage. Trypan blue diffuses readily from the prepared site of cutaneous inoculation and the tributary lymphatics are found unoccluded. *Staphylocoagulase* evidently plays no rôle in inducing early lymphatic blockade. The latter is evidently referable to the powerfully necrotizing action *per se* of the staphylococcus microörganism and of its soluble toxin.

Further studies clearly indicated that the local inflammatory reaction, by determining the degree of invasiveness, plays an important rôle in immunity. The resistance of the host infected with a virulent microorganism represents the resultant of a number of factors, including among others, the invasiveness and the virulence of the bacteria. The invasive property of a microörganism has often been taken as a criterion of its virulence and vice versa (Zinsser, 1931). Some bacteriologists, however, recognize that virulence and invasiveness are different properties which are not to be confused and the terms are therefore not to be employed synonymously (Topley, 1933).

The factor of invasiveness may be studied as a separate variable by a number of experimental procedures. For instance, this was accomplished by interfering with the dissemination of a given virulent microorganism (type III pneumococcus) from its site of cutaneous inoculation (Menkin, 1936 e). This end was attained by the superimposed injection of an inflammatory irritant that causes prompt "walling-off" (e.g., *Staphylococcus aureus*, aleuronat, or turpentine). Such manipulation delayed the dissemination of the virulent microörganism, and even, in some cases, apparently allowed sufficient time for the local inflammatory reaction to dispose of it in large part. In this way the full effect of virulence, as manifested by massive blood stream invasion and subsequent death, could be retarded and in a few cases aborted. Nevertheless,

there was no indication from these observations that the virulence or the toxic property *per se*, of the pathogenic microörganism had been in any way altered by delaying its invasiveness. For this reason it is doubtful whether observations pertaining to virulence and resistance can be adequately utilized in drawing inferences concerning the function of an inflammatory reaction as a regulator of dissemination (Cannon and Hartley, 1938).

In brief, the sum total of all these observations clearly indicates the rôle of the inflammatory reaction in regulating the rapidity of invasiveness of a microörganism, and therefore the significance of this factor in grading the immunity or resistance of the host is manifest. However, the rate of dissemination in no way alters the virulence *per se* of the bacteria, which still remains a most important factor in evaluating resistance. Virulence and invasiveness are therefore regarded as two separate variables in studying problems of immunity (Menkin, 1935 a, 1936 e).

The rapidity and the intensity of local fixation in an inflamed area are factors to be reckoned with in determining the invasiveness of an infectious microörganism. Dissemination seems thus to be related directly to time and inversely to the degree of local injury. This may perhaps be conveniently expressed as follows:

$$D \propto \frac{Kt}{I}$$

where D refers to dissemination, t to time, I to extent of injury, and K is a constant depending on the physical properties of the irritant and perhaps on the anatomical position of the lesion. Powerfully necrotizing irritants produce, as a result of an increase in capillary permeability and lymphatic damage, an extremely prompt "walling-off" reaction (termed *fixation*). By this early process, the area of injury is mechanically circumscribed and the dissemination of the irritant is prevented. This initial response, which may occur within thirty minutes following the injection of an irritant, allows a definite interval of time for the leukocytes to assemble at the site of inflammation for phagocytosis. *Staphylococcus aureus* is an example of such a bacterial irritant. Aleuronat is a chemical irritant of similar potency (Menkin, 1929). Mild irritants, on the other hand, produce only a delayed reaction thus allowing relatively free penetration of the irritant into the circulation for a considerable interval of time. Occlusion of the draining lymphatics in

such instances often takes place as late as two days following the introduction of the irritant. Hemolytic streptococci exemplify this type of irritant (Menkin, 1933 c). Another instance has been recently demonstrated by McMaster and Hudack who showed that, up to 48 hours following a mere skin incision or local burn, lymph drainage is adequate (1934). Subsequently lymphatics failed to convey effectively materials contained in them. The intensity of fixation is found frequently to parallel the extent of inflammatory edema. This, as already mentioned, would suggest that in an inflamed area the local swelling is at least in part the result of blockage to normal lymphatic drainage which is thus unable to cope adequately with the excess outpouring of plasma from the capillaries.

An interesting method for the study of bacterial infection and invasion has recently been devised by Goodpasture and his collaborators (1933, 1937). This consists in inoculating the chorio-allantoic membrane of chick embryos. The tissue behaves as a sterile living culture medium on which various types of bacteria may be inoculated. A means is afforded of propagating pure bacterial cultures in an organism and of thus studying the interaction of host and parasite. Various microörganisms were inoculated, including among others, cultures of *Staphylococcus aureus*, hemolytic streptococci, *C. diphtheriae*, *E. typhi*, and *Myco. tuberculosis avium*. With the exception of *Staph. aureus*, *Str. hemolyticus*, and *C. diphtheriae*, all other pathogens studied were found to multiply readily in the embryonic cells of the host. These microörganisms seem to be able to invade living tissues only through the agency of viable cells. Phagocytosis under these circumstances favors invasion and therefore does not act as a mechanism of defense. These investigators conclude that phagocytosis may thus have an ambiguous implication. It may under some circumstances favor extension of an infection while at other times it tends to eliminate bacteria. These instructive observations indicate the complexity of factors which may be involved in the problem of bacterial invasiveness. It is quite clear, however, from the studies of Goodpasture that in infections due to staphylococci or hemolytic streptococci their invasiveness in tissue is evidently not favored by phagocytosis, for these microbes are destroyed by leukocytes.

There is an additional significant factor that seems to play a rôle in the local dispersion of microörganisms. Duran-Reynals (1933) demonstrated that certain strains of staphylococci and streptococci contain a soluble factor capable of increasing tissue permeability. The filtrates

of these organisms induce a spread of the local lesion. This property is apparently similar to the diffusion of India ink when injected along with testicular extract (Duran-Reynals, 1928, 1929). These observations recall the extensive pneumococcal type of lesion that Goodner described in the dermis of rabbits (1928). He also noticed the presence of an anticoagulating factor in the autolysates of pneumococcus cultures (1931). This property seems to favor the local spread of the edematous fluid containing pneumococci and allows the inoculation of all tissues which it reaches (Rhoads and Goodner, 1931). The writer observed that the injection in the rabbit of broth cultures of type 1 pneumococcus seldom induces any appreciable fibrin deposits in the affected tissue areas. However, the ultimate formation of thrombi in lymphatics prevents the penetration of a vital dye to the tributary lymphatic nodes (Menkin, 1933 e). McClean noted the presence of a similar spreading factor in various members of the gas-gangrene group (1936). Pradhan (1937) likewise confirmed these findings. Some of these investigators conclude that the presence in various pathogens of the spreading factor induces an increase in tissue permeability which in turn seems to be of significance in determining the local invasiveness of a microörganism. They believe that the factor of invasiveness is independent of virulence. This inference, as discussed above, is in agreement with the findings of Menkin (1936 e). In reviewing the various studies on the spreading factor, Duran-Reynals (1936) suggests the possibility that the development of lymphatic blockade may play a part in restricting the full effect of the spreading factor contained in some pathogens. The early "walling-off" induced by staphylococci (Menkin, 1933 c) may thus counteract the effect of the spreading factor which they contain. On the contrary, the widespread types of streptococcal lesions are perhaps referable to the free play of the spreading factor since the development of lymphatic obstruction is considerably delayed. These studies and interpretations are doubtless of great interest. It is questionable, however, in the writer's mind whether the extent of local lesions is of major significance in evaluating invasiveness to the systemic circulation. In the last analysis this seems to be the main problem in regard to the organisms as a whole. Although the extent of local invasiveness is doubtless of some significance, the mechanism of systemic invasiveness seems to be primarily associated with the capacity of microörganisms to reach ultimately the general circulation. As has been noted above, this seems to be a function of the local inflammatory lesion. The patency of the draining lymphatics, through which bacteria disseminate in order

to reach the systemic circulation, is determined primarily by the degree of local injury. Inflammation thus becomes a basic factor in immunity as a regulator of generalized invasiveness.

SUMMARY AND CONCLUSIONS

Inflammation may be regarded as the physical basis of infectious processes. The inflammatory reaction is initiated by disturbances in local fluid exchange. It subsequently proceeds through a series of interdependent sequences which ultimately tend to localize and dispose of the irritant. The disturbances in the local physiology of inflamed tissue are closely associated with the immunological implications of the inflammatory reaction. The principal sequences in the development of inflammation may be listed as follows:

a. *Increased fluid passage through the capillary endothelial wall.* This seems to be primarily referable to two factors: 1. *Elevation in capillary pressure* which is perhaps the outcome of a local axon reflex affecting the caliber of arterioles. 2. *Increased capillary permeability;* this seems to be referable to the liberation by injured tissue of the permeability factor tentatively termed *leukotaxine* (Menkin, 1938 a). Leukotaxine is a crystalline nitrogenous substance, the significant properties of which evidently do not resemble those of histamine. It appears to belong to the group of relatively simple polypeptides. It is postulated that its formation occurs as a result of the presence of an irritant interfering with local protein catabolism.

b. *Localization of the irritant* (fixation). The "walling-off" of an inflamed area seems to be due to an enhanced passage of fibrinogen through the more permeable capillary wall. The mechanism of fixation is primarily referable to the formation of a fibrinous network and of thrombi occluding the lumina of draining lymphatics. This favors the development of lymphatic blockade in acute inflammation. Various secondary factors, such as the presence of immune bodies in anaphylactic or allergic inflammation, may reinforce the basic mechanism. The early occurrence of fixation in a severely injured area plays a definite rôle in immunity, for it allows an interval in which the relatively sluggish leukocytes may assemble for the purpose of phagocytosis.

c. *Migration of leukocytes.* The first cells to migrate into an inflamed area are the polymorphonuclear leukocytes. The mechanism of their migration seems to be related to the liberation of *leukotaxine* by injured tissue. The properties of the crystalline nitrogenous substance recovered from exudates offer a reasonable explanation for two of the

basic sequences of the inflammatory reaction: first, the initial increase in capillary permeability, and secondly, the rapid emigration of polymorphonuclear leukocytes into injured tissue. Leukotaxine appears to contain factors concerned both with permeability and chemotaxis.

The polymorphonuclear cells are gradually displaced by macrophages. Cytological changes in acute inflammation seem to be conditioned by the pH of the exudate which in turn is frequently referable to disturbance in the local intermediary carbohydrate metabolism. The development of a local acidosis, resulting from increased glycolysis and depletion of the alkali reserve, seems to injure the polymorphonuclear cells. Macrophages survive and predominate when the pH falls to a level of about 6.9 or 6.8. Further reduction in the pH proves lethal to all types of leukocytes and frank suppuration ensues. The rôle of lymphocytes or of plasma cells in certain types of inflammation remains to be elucidated.

The interplay and dynamic relationships of the above sequences constitute an acute inflammation. The inflammatory reaction thus displays an extraordinarily complex mechanism tending to localize and dispose of a chemical or bacterial irritant. This ultimately leads to organization and repair of the affected tissue. The concept of fixation and its mechanism affords a rational interpretation of the rôle of inflammation in immunity. The capacity of pathogenic microörganisms to disseminate from their site of inoculation is apparently inversely related to the intensity of induced local injury. In this way inflammation plays a significant rôle in problems of immunity as a regulator of bacterial invasiveness.

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HORMONES AND THE PLACENTA

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At the present time, interest in the placenta as a ductless gland is not so intense as it was a few years ago, and little that is very new can be advanced in this review. The subject is of great importance, and will undoubtedly be re-opened sooner or later. It is now more or less taken for granted that the placenta is an endocrine organ, but its function, except in the most general terms, is not understood. In taking stock of the present position it will become apparent why a straightforward statement cannot be made. There is a plethora of data on the profound changes which take place during pregnancy, pointing more and more to the placenta as the focus, but the unifying conception which would make everything fall into place is elusive.

An attempt will be made to keep as closely as possible to the point, and to remember that this is a review neither of the pituitary nor of pregnancy. Where the findings of different authors are grouped so as to bring them in line with this object, references to their papers will be given separately. It is rarely that different workers make precisely the same observations, but the more nearly these are related, the greater is their tendency to emphasise differences. It is likely that the solution of many problems is to be found in such discrepancies, but in this superficial survey it has been found necessary to ignore them. Although the treatment is in a sense historical, the bibliography is incomplete, there being no limit to the amount of research which bears indirectly on the subject. Human pregnancy and the known hormones have been taken as the basis for discussion.

OESTROGENIC HORMONE. Aschner (1913), Fellner (1913), Hermann (1915) and Schroeder and Goerbig (1921) were among the early workers who demonstrated that human placental extracts, particularly if made with fat solvents, had the power of causing uterine growth in test animals such as the rabbit and guinea pig. All except Schroeder

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and Goerbig used spayed animals, and their experiments were in general an adequate and sometimes striking demonstration of what is now known as oestrogenic material in the placenta. It is obvious, however, that uterine growth is an inconvenient criterion, and Zondek (1931) pointed out that the uterus also responds to such non-specific growth-promoting substances as proteins and amino-acids.

The definite linking by Allen and Doisy (1923) of lipoid ovarian (follicular) extracts with the observed activity of the living ovary in relation to oestrus crystallized the conception of a "follicular hormone." The simple specific test for this was vaginal cornification in the spayed mouse or rat, and the substance was at once sought in various parts of the female genital tract. It was re-discovered in the human placenta by Doisy, Ralls, Allen and Johnson (1924), Zondek and Brahn (1925), Frank and Gustavson (1925) and Dickens, Dodds and Wright (1925), while Allen, Pratt and Doisy (1925) showed its presence in a chorionic vesicle of the second month of pregnancy. Later it was found by Parkes and Bellerby (1927) in the placentae of the cow and sheep, and in the same year by Allen (1927) in both full term and earlier placentae from the cow and in full term chorions from the mare. Allen, Maddux and Kennedy (1931) showed it to be present in small quantities in the placenta of the macaque and Allen, Diddle and Elder (1935) found larger amounts in the placenta of the chimpanzee. Allen (1925, 1927) obtained negative tests from the placentae of the pig, dog, cat and rat, while Philipp (1929) using implants reported positive results for pig but negative for rabbit placentae. D'Amour, Funk and Glendenning (1936) also failed to find oestrogenic activity in rat placentae.

In the isolation of pure oestrogenic substances, the placenta was largely neglected, but crystalline oestriol was obtained from it in Collip's laboratory in 1932. Two years previously Collip (1930) had reported a water-soluble, ether-insoluble, oestrogenic principle in the human placenta to which he gave the name "emmenin." It was characterised by greater potency in immature than in adult spayed test animals, and this, as well as its solubility, differentiated it from oestrone. Its physiological properties, apart from its oral activity and low comparative potency, were the same as those subsequently found for oestriol (Marrian, 1930) and for an ether-soluble crystalline product from the placenta (Collip, Browne and Thomson, 1932) which was later proved to be oestriol (Butenandt and Browne, 1933). In 1934 Collip, Browne and Thomson formally withdrew the name "ether-soluble emmenin" for this second compound. Emmenin itself, on acid hydrolysis, yielded

an active substance which was ether-soluble, and of which crystals were obtained. This was presumably oestriol, and the water-solubility of the parent substance was attributed to its being a compound of oestriol with some inert substance (see Collip, Browne and Thomson, 1934). This is probably glucuronic acid. Oestriol glucuronide, containing 50 per cent oestriol, has been isolated from the urine of human pregnancy (Cohen and Marrian, 1935; Cohen, Marrian and Odell, 1936), and Odell, Skill and Marrian (1937) have shown that, like emmenin, its potency when given orally to test animals is extraordinarily high compared with its potency when injected, the ratio being approximately 5:4. The ratio of activity of orally administered oestriol to that of orally administered oestriol glucuronide is, however, about 2:1, in accordance with the composition of the latter, and an intestinal glucuronidase has been demonstrated. The oestriol liberated by hydrolysis in the intestine, although only one-tenth as potent as the same quantity injected subcutaneously, exhibits the same order of oestrogenic activity as the injected unhydrolysed glucoronide. Human placenta therefore contains at least two oestrogenic substances—oestriol and emmenin, the latter being almost certainly oestriol glucuronide. Collip (1932) states that oestrone is also present, though not actually isolated, and Westerfield and Doisy (1937) have confirmed the presence of ketonic oestrogen.

It is difficult to make any final statement about the quantitative oestrogenic activity of human placental tissue. The values given by different workers range from 500 to 5,000 mouse units (M.U.) per whole placenta, and these may equally well indicate individual variations in the placentae or in the methods of assay. Values between 1,000 and 2,000 M.U. are more common (Allen, Pratt and Doisy, 1925; Philipp, 1929; Butenandt, 1931; Zondek, 1931; Smith and Kennard, 1937). Making fairly generous allowance for experimental error, and assuming an average placental weight of 500 grams, Clauberg (1936) calculates that placental tissue contains at least as much and probably more oestrogenic material per gramme than the ovary. If this is so it is a point in favour of its secreting oestrin, or actively removing it from the blood stream, but it is well to bear in mind that Catchpole and Cole (1934) found the kidney to have a higher oestrogenic activity than any other tissue of the pregnant mare, and to accept with reserve arguments for secretion based on local concentration.

The most important finding in favour of secretion of oestrin by the human placenta is the excretion of the hormone after double oöphorec-

tomy in early pregnancy. The evidence, though positive, is disappointing. It is worth dealing individually with the small number of cases quoted (e.g., by Clauberg, 1936) in support of this theory. Amati (1928) injected into immature guinea pigs serum taken on the third day of the puerperium from a woman oöphorectomised in the third month of pregnancy. The accessory genital organs showed typical oestrous changes, but the ovaries were also observed to have been stimulated. This case, therefore, may be entirely neglected. Waldstein, (1929) gives an estimate of 400 to 500 M.U. of oestrin per litre of urine, about four weeks before delivery, from a woman oöphorectomised when approximately two months pregnant. It is not clear that any other urine test was performed, except for one in the puerperium which gave a smaller numerical result. The placenta contained about 300 M.U. Szarka (1930) found 1000 M.U. per litre of urine about 10 hours before parturition in a woman oöphorectomised when about three months pregnant. Saidl (1932) quoted by Allan and Dodds (1935) is said to have obtained results similar to those of Waldstein. The case reported by Allan and Dodds themselves was not followed to full term, but while 2,000 M.U. per litre were found before oöphorectomy (at about three months), only 1300 M.U. were present three months later. This was about the same as the immediate post-operative value. The remaining two cases are quite different in that higher values were found, and were compared with assays made on the urine of normal patients by the investigators themselves. After oöphorectomy in the second month, Probstner (1931) estimated the oestrin content of his patient's urine every three or four weeks—the most complete study recorded. The hormone virtually disappeared from the urine for eight weeks, after which it rose fairly rapidly to a value of 7,000 M.U. (probably per litre) in the sixth month and remained at this height, or somewhat higher, till full term. Exact values are not given, but the smoothed curve falls almost exactly on the smoothed curve given for a normal patient during the last three months. Brindeau, Hinglais and Hinglais (1934a) report values of 1200 R.U. (approximately 6,000 M.U.) in the last month of pregnancy from a patient from whom one ovary was removed at the third month, the other having been removed at some previous time. They remark that their results are conditional upon the oöphorectomy having been complete. The figures given compare well with those given for normal patients.

These results show that some extra-ovarian source of oestrin was active during the last two-thirds of pregnancy, but further interpreta-

tion is difficult. Except for the last two cases, the values are very low by any standard, but in these two, though the values are also low, they must be admitted as "normal" by the standards of those reporting them. After the urine has been hydrolysed, the oestrin content in the last month of normal pregnancy may rise to about 70,000 R.U. or 350,000 M.U. per litre (Cohen, Marrian and Watson, 1935; Smith, Smith and Pincus, 1938), and these may be taken as normal values. But in their earlier work, Smith and Smith (1935b) find that hydrolysis may increase the activity of the urine 40 times or only by one-third, so a standard cannot be fixed for unhydrolysed urine. If the figure be divided by 12 according to the data of Odell, Skill and Marrian (1937) on the activity ratio between oestriol glucuronide and oestriol, a "normal" value of about 30,000 M.U. is obtained which agrees roughly with Zondek's criterion (1931 b) of 20,000 M.U., obtained by the method of direct injection. If we accept the low values obtained for urinary oestrin after oöphorectomy, and assume that they differ from the normal by the normal ovarian output, it can be computed from the data given by Smith, Smith and Pincus for non-pregnant women that the activity of the ovaries is increased about 300 times during pregnancy. Frank, Goldberger and Salmon (1936) and others have demonstrated that the urine of non-pregnant oöphorectomised women is oestrogenic, and Callow and Parkes (1936) have found that extracts of animal suprarenal and pituitary have oestrogenic activity. If an extra-genital source of oestrin increased its activity by 300 times during pregnancy, it would be more than sufficient to account for the small oestrin excretion found by Waldstein and others after oöphorectomy. These considerations are introduced to show that the existing data are subject to more than one interpretation.

According to Probstner, no contribution by the ovaries need be assumed during the last months of pregnancy, but his results suggest an entirely new consideration. After the operation, oestrin is absent from the urine at a time when it is high in the normal individual, and its slow recovery strongly suggests a compensatory mechanism, i.e., the extra-ovarian source of oestrin may be more active in the absence of the ovaries than when they are present.

It is generally assumed that the extra-ovarian source is the placenta, and its normally high content supports this. Another important point in favour of it is that in oöphorectomised non-pregnant women the accessory organs atrophy. The factor preventing this during pregnancy is something associated with pregnancy itself, and the simplest explana-

tion is that this is the placenta. It is of great importance that a further case of oöphorectomy during pregnancy should be investigated by modern methods.

The researches of Cole and his associates on the pregnant mare are much more satisfactory, and can therefore be treated briefly. Unfortu nately, they cannot be held as more than suggestive as far as man is concerned. The main facts are as follows: The mare's urine is oestrogenic during pregnancy, reaching its maximum activity between the 200th and 275th days. Three mares followed throughout pregnancy gave at this time maximum values of 17,000, 17,000 and 33,000 R.U. per litre respectively (Cole and Saunders, 1935), which agree well enough with Zondek's figure of 100,000 M.U. A mare spayed on the 200th day yielded about 10,000 R.U. per litre of urine during this period and foaled normally on the 350th day (Hart and Cole, 1934). A generous calculation shows that the total quantity of oestrogenic material excreted after oöphorectomy was far in excess of what the body could have contained at the time of operation. It was closer to the normal than the figures just quoted indicate. An extra-ovarian source of oestrin is therefore proved in the mare, and the quantity eliminated is nearer to the normal than it appears to be in the human. The greatest concentrations of oestrogen in the body of the pregnant mare have been found in the kidney, endometrium and placenta (Catchpole and Cole, 1934), and the placenta is regarded as the source. The ovaries of the mare are fibrous with few follicles, in the last third of pregnancy (Cole, Howell and Hart, 1931) and although the foetal gonads exceed them in size at one time, the concentration of oestrin in the placenta is greater than in any foetal tissue.

Philipp (1929) states that implantation tests reveal no activity on the part of the ovaries of the sow in late pregnancy, but that the foetal part of the placenta (readily separable in this animal) yields positive tests. Küst (1934) states that this animal excretes oestrin in the urine when pregnant. Other animals whose urine becomes oestrogenic during pregnancy are chimpanzees (Allen, Diddle and Elder, 1935); macaques (Allen, Maddux and Kennedy, 1931), cows (Aschheim and Zondek, 1927; Hisaw and Meyer, 1929; Nibler and Turner, 1929; Lipshutz and Veshnakow, 1930), goats (Küst, 1934); and rats (D'Amour, Funk and Glendenning, 1936). Philipp (1929) claims to have shown it in the blood of rabbits toward the end of pregnancy.

The oestrogenic activity of blood during pregnancy is of importance, but a discussion of the findings of various workers would add nothing

of material value to this review. The reader is referred to that of Frank (1935). It should be mentioned that when tested, the blood of oöphorectomised pregnant women was found to have a normal content.

Smith and Smith (1935 c) failed to demonstrate oestrin in the blood of 4 patients with chorionepithelioma, 3 of whom were males. The urine had to be concentrated before it was possible to show 100 R.U. or less in 24 hours, and could not be regarded as other than normal. The intense interest attached to determinations of gonadotropic hormone values in chorionepithelioma has led to the neglect of oestrin, so much so that Smith and Smith state that they were unable to find previous data. A case is, however, recorded by de Snoo (1928) in which a true chorionepithelioma of the tube together with the uterus and both ovaries, was removed surgically from a woman. Three months later the patient returned with metastases, and Laqueur showed that the urine contained 300 to 1000 M.U. of oestrin. In a similar case, though in a child of six, Siegmund, (1932) was unable to demonstrate oestrin, though large amounts of gonadotropic material were present. Fels (1930) was able to show 1 M.U. of oestrin in 1.5 to 2 cc. of fluid from a hydatidiform mole, though the patient had none in the urine, and also 1 M.U. in 4 cc. urine from a male patient with chorionepithelioma of the testes (see also Heidrich, Fels and Matthias, 1930). These urine values are very small compared with those found in pregnancy when the placenta is attaining its full size, but they recall those reported in pregnancy after oöphorectomy. Owing to the presence of gonadotropic hormone, de Snoo's case is of the most significance since his patient had no gonads.

The balance of evidence at present indicates that the placenta can produce oestrin, but there is little to show that it is responsible directly for the enormous amounts found in human pregnancy. The writer is not convinced that in the mare the hypertrophied foetal gonads, both types of which seem to contain oestrogen, may not contribute materially to the urine of that animal after oöphorectomy (see Cole, Hart, Lyons and Catchpole, 1933; and Brill, 1933). The demonstration of a source of oestrin outside the genital tract which could be stimulated to greatly increased activity would impair the case for the placenta.

THE HORMONE OF THE CORPUS LUTEUM. Clauberg (1936) dismisses the placenta as a source of progestin on the ground that after the third month the products of gestation in man are self-supporting, so no further "progestational" changes are necessary. The fact is proved by the now numerous reported cases in which oöphorectomy

has been performed early in pregnancy without interrupting it. The commoner inference is that since in most animals the corpus luteum is necessary throughout gestation, those animals, such as the guinea pig, horse and man, which can dispense with it must have some other source of progestin. Is the source the placenta?

The evidence at present is so scanty that it requires merely to be recorded. The most significant experiment is that of Haterius, (1936) who removed all except one of the fetuses from the pregnant rat, leaving the supernumerary placentae attached to the uterus. On oöphorectomy, his animals did not abort but maintained pregnancy to full term. This is clear evidence that the placentae successfully replaced the ovaries up to the time for parturition, which, incidentally, did not take place.

Selye, Collip and Thomson (1935) on the basis of the histological appearance of the mammary gland and uterus of the oöphorectomised rat, with retained placentae, also postulate secretion of progestin by the placenta. Klein (1935) has shown that as far as the vaginal epithelium of such rats is concerned, a distinct change suggesting withdrawal of progestin takes place on oöphorectomy. But if a division of labour is to be assumed between ovary and placenta, we should expect to find only small amounts secreted by the placenta of the rat, whose ovaries are normally essential throughout pregnancy.

The human corpus luteum contains very little progestin, about 1/30 that of the sow, though this does not necessarily mean that it secretes less. It is, however, consistent with the unimportance of the human ovary in pregnancy. Progestin has been demonstrated in the human placenta (Ehrhardt, 1934; Adler, Fremery and Tausk, 1934; McGinty, McCullough and Wolter, 1936; Ehrhardt and Fischer-Wasels, 1936; Smith and Kennard, 1937), but the quantity is not large. Much more significant are the isolation by Venning and Browne (1936) of a pregnandiol-glucuronate compound from human pregnancy urine, the close association of its excretion in non-pregnant women with the luteal phase of the menstrual cycle, and its recovery from the urine after injecting progesterone (Venning and Browne, 1937). Browne, Henry and Venning (1937) state that this compound is present in the urine in amounts normal for the menstrual cycle (4–10 mgm. p.l.) up to the 60th day of pregnancy when it begins to rise, attaining 40 mgm. per l. by the 150th day and 70 to 80 mgm. per l. by the eighth month. By 24 hours after parturition it has disappeared from the urine. (Since some of these data have been obtained from preliminary communications, the

writer presumes that further papers will have appeared on the subject by the time this review is published.)

GONADOTROPIC HORMONE, A.P.L. Aschner (1913), and Hermann (1915), described the ripening of numerous Graafian follicles as one of the sequels to injection of placental extracts into young test animals, and the former remarked that uterine changes were much more marked when these were intact than when they were spayed. Hirose, (1920); Adachi, (1924); and Murata and Adachi (1927) described luteinisation of follicles in addition, and the last authors showed that extracts of hydatidiform mole and chorionepithelioma had the same action.

Meanwhile, Evans and Long (1921) in their work on the growth hormone had shown that after prolonged injections of an anterior pituitary extract into rats, the ovaries were luteinised and greatly increased in size and the oestrous cycles were suppressed. Zondek and Ascheim (1926, 1927 a, b) and Smith (1926, 1927 b; Smith and Engle, 1927) added the crucial observation that the immediate effects of implantation of pituitary tissue into immature rodents included the ripening and ovulation of an enormous number of Graafian follicles, and Ascheim and Zondek (1927) showed later that injection of blood or urine from women in early pregnancy was followed by the same result; luteinisation also occurred. A complete positive Ascheim-Zondek (A-Z) pregnancy test (Ascheim and Zondek, 1928) requires the appearance of ripe follicles with oestrus, blood follicles and corpora lutea.

These fundamental observations aroused great interest and opened up several lines of investigation. The anterior pituitary, removal of which is followed by atrophy of the gonads and a secondary atrophy of the accessory genital organs (Smith, 1927 a) is undisputed as the proper source of the gonadotropic hormone or hormones which control the reproductive organs and which, when administered in excess, produce the ovarian effects just described. The question which concerns us is whether placental tissue itself secretes the substance which lends it similar properties to that of pituitary tissue when it is implanted into or administered in the form of extracts to test animals. It may be stated at once that there is no answer to this question based on strict logical reasoning: indeed, there is a strong case for its being unanswerable, for hypophysectomy is unlikely to be performed on a pregnant woman and would in any case almost certainly terminate the pregnancy. It is only possible therefore to survey such facts as at present seem relevant.

Zondek (1931 b) on the basis of the least weight of implanted placental tissue (ether-extracted) which would elicit oestrus, with ovarian blood follicles and luteinisation, in the immature mouse, found that a 7-week placenta contained 1,144 M.U., an 11-week placenta 1,132 M.U., and a full term of placenta 5,800 M.U. of A.P.L. The respective weights of these human placentae were 8, 34 and 580 g., so that the concentration of A.P.L. was considerably less at the end of pregnancy than in the early stages. Philipp (1930 a), implanting samples of equal size from different placentae also concluded that, gramme for gramme, earlier placentae were more potent than later ones. He also stated (1930 c) implants of early placentae caused luteinisation rather than follicle-ripening, but that such activity as was displayed by full term placentae led largely to follicle-ripening. These conclusions were reiterated later (Philipp, 1931), the rabbit's uterus being used as a test for the secondary production of progestin. Zondek (1931 a) criticised Philipp's technique, remarking that potent implants tended to inhibit follicle-ripening and cause excessive luteinisation, whatever their relative content of the two (assumed) active principles. He himself always implanted a series of mice with a complete range of samples of ascending weight from each specimen of tissue to be tested, thus finding the threshold "dose."

Philipp has ardently sponsored the placenta as truly producing gonadotropic hormone, the mainstay of his argument being the absence of gonadotropic activity in the pituitary of pregnant women when implanted into test animals (Philipp, 1930 a, b, d; 1931). This has been amply confirmed (Zondek, 1931 a; Ehrhardt and Mayes 1930; Saxton and Loeb, 1937, and others). On the basis of a large number of implantations of human pituitary Philipp (1930 d) found that the pituitaries of children before puberty gave a follicle-stimulating reaction which gradually intensified with age, and that the adult pituitary at all ages, but not in pregnancy, gave the full picture of follicle stimulation and luteinisation. He correlates the follicle stimulating power with the content in eosinophile cells, (disputed by Zondek, 1933) but his contention that the pituitary after oöphorectomy causes practically no luteinisation (1931) is rendered doubtful by the post-menopausal cases he himself and the other investigators report in which a complete A-Z reaction was obtained. In other words, the behaviour of the pituitary on implantation does not accord very well with that of the urine of the same period of life—at this time purely follicle-stimulating.

Motta (1930), though producing no experimental evidence, is interesting in that he puts forward a very plausible argument from the same

data that the content of the gland is in inverse proportion to its activity, and that the chromophile cells, relatively few in pregnancy, are storage cells. Zondek (1931 a, b) takes up a cautious attitude, pointing out that the mere relative content of an organ in active material means little. He cites the thyroid in Graves' disease, and an ovarian cyst which he happened to find with a gonadotropic power in excess of that of the placenta. We have previously remarked on the high oestrin content of the mare's kidney in a similar connection. There can be no doubt that the absence of gonadotropic activity and the well known changes in the human pituitary of pregnancy are closely related to the problem, but in what way we have no means of knowing. It is possible that the characteristic cytology is caused by high concentration of oestrin in the blood, but it is not legitimate to dismiss it as purely incidental on this account as Philipp is inclined to do. Victor and Andersen (1937) find, for instance, that oestrin accelerates the metabolic rate of the animal pituitary, which is in accordance with the stimulating effect described by Fevold, Hisaw and Greep (1936 a).

The association of trophoblastic tissue with large quantities of gonadotropic substance in the body is unmistakable. Not only does the latter rapidly disappear from the urine and blood after parturition, but in cases of incomplete abortion it persists until all placental remnants have been delivered (e.g., von Arvay, 1934). Ware and Main (1934) describe a case of abdominal pregnancy in which the foetus was removed about a month before full term, the placenta being left attached to various abdominal viscera. Gonadotropic hormone was excreted in the urine for 36 days. Frank (1929) has reported a similar case.

More striking is the production of gonadotropic substance in cases of hydatidiform mole and chorionepithelioma described by many early observers (see Zondek, 1931). Seigmund's (1932) case of a child of 6 with metastases, from whom the original focus had been removed, is sufficient to show that the production is independent of the reproductive tract and of the sexual maturity of the patient. Heidrich, Fels and Matthias (1930) found large quantities in the urine of a man with metastases from a chorionepithelioma of the testis which had been removed some weeks earlier, thus establishing the absence of sex-specificity. It is worth remarking, that the anterior pituitary gland of this patient had a structure typical of that of pregnancy, and gave a negative A-Z reaction on implantation. Nevertheless, only 350 M.U. per litre of oestrin were demonstrated in the urine (one testicle remained). The hypertrophy of the mammary gland, which contained

colostrum, and the hyperplasia of the prostate and seminal vesicles are of interest, but cannot be discussed here: the carefully recorded findings in this case are provocative in many ways. The rate at which gonadotropic hormone is excreted in chorionepithelioma is extremely high and now forms the basis for differential diagnosis (see Brindeau, Hinglais and Hinglais, 1935, and Clauberg, 1936).

Philipp and Huber (1936) have recently advanced new evidence in favour of the actual production of gonadotropic hormone by the placenta, on the basis of tests on the decidua in abortions and abnormal types of pregnancy. The decidua gives a positive reaction only so long as placental tissue is present, but is negative if remote from the placenta as in the non-gravid horn of a bicornuate uterus, or the uterus of a tubal pregnancy. In other words, it has no concentrating power of its own, and if the placenta were merely collecting hormone from the bloodstream the content of the decidua should be unaffected. On the other hand, if the placenta secretes the hormone a local concentration in the decidua is readily explained. This evidence is clearly not conclusive, since other explanations are possible.

The most promising advance towards a final solution of the problem has been made by Kido (1937) who states that he has obtained survival of human chorionic villi when transplanted into the anterior chamber of the eye of the rabbit. The amount of tissue transplanted was less than that required to produce an ovarian response when implanted intramuscularly or extracted and given intravenously, yet a positive reaction was obtained and the urine of the host was active when injected into a second rabbit. Taking into account the absence of immediate autolysis and the possibility of the site as a favourable one for rapid absorption, it cannot be said that the pre-existing hormone is absolutely excluded, but activity of the urine on the second rabbit is more convincing. It is a pity that the time relations of these events are not given more clearly. Accepting the experiments at their face value, the significant question is whether the small transplant is itself producing the hormone or whether it is stimulating the rabbit's own pituitary. To settle this point it should be only necessary to repeat the experiment with suitable precautions in hypophysectomised animals.

Apart from this last development, which has been inserted at this point for convenience, the type of evidence we have been considering, though suggestive, promises always to fall short of actual proof. The case for placental secretion on such grounds has been well put by Fels (1932), Clauberg (1936) and repeatedly by Philipp (1929, 1930, 1931,

1932): these authors give more numerous citations from the literature than are possible here, and advance or review certain theories. One of these theories (Clauberg), whose actual origin is indefinite, is that the mature ovum itself produces a "gonadotropic" substance determining the formation of the corpus luteum and that after fertilisation its output is increased, thus inducing the corpus luteum of pregnancy and finally resulting in the enormous output of the early months of pregnancy. Both Westman (1934) and Zondek (1934) by removing the ova from ripe follicles in the rabbit have shown that corpus luteum formation is independent of the ovum and Philipp (1930) was unsuccessful in obtaining a positive Z-A reaction from ripe human follicles. Von Maroudis (1933) has, however, reported the production of haemorrhagic follicles and corpora lutea in two out of three cases in which very early fertilised human ova, two or three weeks old, were implanted into rabbits. Fertilization of the ovum is undoubtedly responsible in some way for the corpus luteum of pregnancy, and the second part of the theory, suggesting that gonadotropic hormone formation in the egg dates from fertilisation, is very plausible. As before, actual proof of production in the egg is lacking, but it must be admitted that the alternative theory that the fertilized ovum first stimulates the pituitary and then stores the pituitary secretion at this early stage appears clumsy.

The significance of comparative data is at present obscure. Philipp (1929) stated that the foetal part of the pig placenta gave a positive A-Z reaction; Menzani and Gentile (1934) have reported positive, though irregular, tests from the urine of cows between the 38th and 150th days of pregnancy; and the existence of a gonadotropic substance in the serum of the pregnant mare (Cole and Hart, 1930; Zondek, 1930; Glud, Pederson-Bjergard and Portman, 1933; Catchpole and Lyons, 1934; Cole, 1936; Rowlands, 1938) is well established. Catchpole and Lyons (1934) find the hormone also in the allanto-chorion and decidua of the mare, and, like Glud et al., draw attention to the fact that it suddenly appears in the blood at the time when the maternal and fetal tissues first make definite contact. In man, the studies of Browne and Venning (1936) and Evans, Kohls and Wonder (1937), show that the sudden appearance and rise in concentration of gonadotropic hormone in the urine occurs between the 40th and 50th days of pregnancy (counted from the last menstruation). This corresponds approximately to the time at which the foetal circulation is established in the chorionic villi. In the mare, the hormone, after rising to high levels, disappears from the blood after about 100 days. In the pregnant

woman it drops to a low level between the 60th and 80th days, but persists till after parturition. In the urine of the chimpanzee it appears from the 95th to the 100th day of pregnancy (counted from last menstruation) and persists for 50 to 60 days (Shultz and Snyder, 1935). In the macaque monkey, Hamlett (1937) has found it only between the 19th and 25th days of pregnancy. The writer has been unable to ascertain to what these times correspond in the development of the monkey and chimpanzee placentae, but Hamlett correlates them with the "establishment" of the placenta. At the moment it seems inadvisable, in view of the transient appearance of hormone in the macaque, to stress the negative results which have been reported for other animals. In the case of large animals it is possible that sufficiently systematic and continuous blood and urine examinations have not been made, while in small animals the quantities of hormone may be too little for detection, or it may only appear for a few hours.

Nevertheless, Emery (1932) has been able to show the presence of a gonadotropic substance in the blood of castrated rats, which argues that the size of the animal is not an insuperable obstacle. The pregnant mare and cow show that the production of gonadotropic hormone during pregnancy is not confined to animals with haemochorial placentae, and although the hormone of the mare is of a different variety from that excreted in human pregnancy, and has a pronounced effect on the maternal ovary, one cannot help but feel that the species differences may conceal some rational evolutionary development. So far, the state of affairs in other animals does not help us a great deal with the subject under discussion.

In 1931, M. Hill and Parkes pointed out that testing for the anterior pituitary-like principle while the gland of the test animal itself was in place was equivalent to testing for oestrin in the presence of the ovaries. It is strange that so much controversy should have centered round the question whether the substance in the urine of pregnancy was of placental or hypophyseal origin when there was no rigid proof that it had any anterior pituitary-like properties at all: it might have been a substance with no action on the ovaries except through indirect stimulation of the pituitary. It is true that immature animals were used in the A-Z test, but the result of a positive test in itself proves the capacity of infantile tissue to react to stimulation. Before the sudden extension in the use of hypophysectomised animals took place, there had already been strong indications that the gonadotropic substance in the placenta,

blood and urine in pregnancy was not the same as the gonadotropic substance of the hypophysis.

Leonard, (1932 a); Collip, (1932); Fluhmann, (1932); Hamburger, (1933) and Smith and Smith (1935 a) state that the gonadotropic effects of placental extracts are the same as those of pregnancy urine (P.U.). The differences found between the actions of anterior pituitary (A.P.) and P.U. or placental extracts may be summarised as follows:

1. *Injurious or negative effects of P.U. on the ovaries, particularly the follicles, as contrasted with a stimulating effect by A.P.* Watrin and Brabant, (1931), infantile rabbit; Bourg (1931), cat; Engle (1933, 1934), Johnson (1935), monkeys; Geist (1933), human.

2. *Differential effects on the ovaries of immature mice or rats.* Philipp (1930 b); Evans, Meyer and Simpson (1932); Fluhmann (1932, 1933); Hamburger (1933); Leonard (1933 a); Smith, Engle and Tyndale (1934); Brindeau, Hinglais and Hinglais (1934 b); Rowlands (1935); Deanesly (1935). It would require too much space to describe in detail the findings of these and other authors, influenced as they are by different methods of administration, different experimental periods and difference in the age of the animals. It is difficult also to summarise them, but the general tendency is to show that A.P. has a greater stimulating effect on more follicles (many observers report negative results with P.U.) and increases the size of the ovary in proportion to dosage. P.U. increases ovarian weight only to a certain maximum. The secondary effect of P.U. on the size of the accessory genital organs is greater than that of A.P.

3. *Relative threshold differences, in different animals,* the doses for equivalent effects of the two substances on one animal (e.g., the production of oestrus, or a given increase in ovarian weight) being used as a standard of dosage for the second animal—Wallen-Lawrence and van Dyke (1931), male and female rats; Leonard (1932 b, 1933 a), Mahnert (1933), rat and rabbit; Hamburger (1933) rat and mouse.

4. *Differential effects on immature male genital organs with regard to spermatogenesis, interstitial cells and secondary sexual characteristics.* No qualitative difference in rats and monkeys (Engle 1932) but a different ratio of effect on the weight of accessory organs and testes in rats (Schockaert 1933); a difference in relative activity on epithelial and muscular components of the accessory organs (Freud and de Jongh, 1933). In immature birds, P.U. has no action on the testis, while A.P. causes hypertrophy of interstitial tissue and growth of accessory

organs (Reiss, Pick and Winter, 1933; Pompen, Dingemanse and Kober, 1933; Freud, 1933 b; Schockaert, 1933).

5. *The effect on hypophysectomised animals.* M. Hill and Parkes (1931) had initial difficulty in inducing ovulation with P.U. in hypophysectomised rabbits, and White and Leonard (1933) found that although the minimal dose for ovulation was increased comparatively little after hypophysectomy there were differences between A.P. and P.U. Reichert, Pencharz, Simpson, Meyer and Evans (1932) reported that P.U. was ineffective on the ovaries of rats and dogs except after prolonged treatment with large doses. Collip, Selye and Thomson (1933) found that gonadotropic placental extract, while acting on the interstitial cells of the testes of male rats, failed to support the germinal epithelium after hypophysectomy. Although oestrus was produced in mature hypophysectomised females, it failed to appear after treatment in infantile animals. This was in contrast to the complete replacement by A.P. (Smith, 1927 a). Wade, Kalzman and Jorgenson (1933) obtained similar results with immature females, and Smith and Leonard (1934) and Leonard and Smith (1934 b) with both sexes (except for some stimulation of germinal cells in the male). In mature animals of both sexes, Smith and Leonard (1933) and Leonard and Smith (1933 a) obtained sufficient gonadal stimulation to produce fertile mating, but an absence of follicle-stimulation (excluding ovulation) in the females and a subnormal effect in males (compared with A.P.) were noted. Without attempting to do justice to differences of opinion on certain points, it may be said that these experiments prove P.U. to be gonadotropic, but to replace A.P. incompletely in the absence of the test animal's own pituitary. Its deficiencies are much less marked the longer the ovary or testis has been under the influence of the anterior pituitary, i.e., the older the animal when hypophysectomised. Its characteristic action is to produce thecal luteinisation (Selye, Collip and Thomson, 1933 b; 1934; Freud, 1933 b; Leonard and Smith, 1933 b, 1934 b).

6. *Potentiation effects.* The significance of such observations is emphasised by the fact that numerous experiments on immature and hypophysectomised animals have shown that minimal or subliminal doses of P.U. combined with similar ineffective doses of an A.P. principle will produce far greater effects than can be accounted for by the sum of their separate effects. At this point it is necessary to mention the successful separation by Fevold, Hisaw, Hellbaum and Hertz, (1933 a) and Wallen Lawrence, (1934) from pituitary extracts of follicle stimulating (F.S.H.) and luteinising (L.H.) principles, named after their typical actions on the

ovaries of immature or hypophysectomised rodents. The former authors (1933 b; Fevold and Hisaw, 1934) quickly showed that L.H. + F.S.H. yielded an increase in weight of the ovaries of immature rats beyond the separate action of either, that a similar synergism existed between F.S.H. and P.U. (or placental extract) but not between L.H. and P.U. A similar synergism was demonstrated by Leonard and Smith (1933 b, 1934 a) between P.U. and the gonadotropic principle (F.S.U.) from postmenopausal urine, or urine of either sex after removal of the gonads, which itself has an almost purely follicle-stimulating effect in hypophysectomised animals.

These observations have an obvious bearing on the description by Evans and his associates of a remarkable potentiating action on P.U. by small quantities of a "synergist" extracted from the anterior pituitary. This was at first thought to be associated with the growth hormone, was obtained independently of this, was claimed to have no independent and then slight gonadotropic activity, and was declared by Fevold and Hisaw (1934) to have a mixed gonadotropic effect (Evans, Meyer and Simpson, 1932; Evans, Simpson and Austin, 1933 a, b; Evans, Pencharz and Simpson, 1934). It is doubtful if Fevold and Hisaw completely explained away the result of Evans et al., but the claims of the latter, particularly to have unmasked gonadotropic activity in tissues and animals hitherto negative (Evans, Simpson and Austin, 1933 c), must be viewed in the light of work by Saunders and Cole (1936) who find that the synergist is itself activated by such non-specific substances as zinc sulphate, egg albumin and casein (see also Fevold, Hisaw and Greep, 1936 b; Emery, 1937; McShan and Meyer, 1937). Apart from a statement by Collip, Selye, Thomson and Williamson (1933) that a potentiating substance obtained from the pituitary was probably not identical with any of the known hormones, most of the facts relating to potentiation can be explained on the grounds of non-specific activation or a complementary action between F.S.H. and L.H. or their equivalents (see also Smith, Engle and Tyndale, 1934; Hertz and Hisaw, 1934; Leonard, 1934 a; Engle, 1934). In experiments on intact immature animals the possibility arises of actual stimulation of the animals' own pituitary by injected P.U., but whereas the pituitaries can be shown on histological examination or by subsequent implantation into further animals to have been influenced, it has been repeatedly observed that such deviations from the normal are not found in the absence of the gonads (e.g., Leonard, 1933 b; Severinghaus, 1934 a; Wolfe, 1934).

De Jongh and Kober (1933) have described somewhat different potentiating experiments. They state that in the spayed immature rat, the action of oestrin is potentiated by A.P., but not by P.U., so that in the intact animal the oestrin released from the ovary by A.P. would be potentiated, thus giving an apparently high potency. Freud (1933 a) has described parallel effects in the male.

It is now generally taken for granted that the gonadotropic principles of the placenta, blood and urine in pregnancy are identical, and differ in their action from anterior pituitary extracts because they contain only the luteinising and not both the follicle-stimulating and luteinising principles. The gonadotropic substance appearing in the functional or actual absence of the gonads, and in the case of some non-chorionic tumours is thought to be deficient in the luteinising principle, and not completely identical with the secretion of the anterior pituitary as assumed by Hamburger (1933), Leonard and Smith (1934 a).

It is doubtful if the state of affairs is as simple as this. F.S.H. and L.H. are usually products of sheep or ox pituitary, and their nomenclature depends on their action on the immature rat and mouse. Not only do the pituitaries of different animals vary greatly in potency in ordinary tests (see e.g., Magistris, 1932), a fact which may be merely incidental, but when the conventional test animals are left and the pituitaries of various species are administered to various test animals, the subject relapses again into confusion. For instance, the pituitaries of rabbit, rat and guinea pig cause follicular growth in guinea pigs, but those of the ox and sheep cause follicular atresia (Aff and Loeb, 1934). In the hypophysectomised ferret, A.P. causes luteinisation of small follicles and no obvious change in the accessory organs, while P.U. causes marked follicular growth, culminating in atresia, and oestrous swelling of the vulva in the first phase. A combination of A.P. and P.U. failed to produce full oestrus, and in 5 out of 6 cases, no follicular growth (McPhail, 1933). This is almost precisely the opposite result to what might have been expected. Until recently it was assumed on the grounds of implantation experiments in rats and mice that oestrin depresses and oöphorectomy releases the pituitary, but if ovulation in the rabbit is used as a criterion, the reverse result is obtained (R. T. Hill, 1934). There seems to be no parallelism between the potency of different pituitaries expressed in terms of their activity in different test animals (R. T. Hill, 1934; Rowlands, 1935). The frequent postulation of "third" principles with various effects is a symptom that the final solution is yet to be reached (see e.g., Leonard, 1934 b; Lipshültz, 1934,

1935 a, b; Leonard, Hisaw and Fevold, 1935; Evans, Korpi, Simpson, Pencharz and Wonder, 1936). Bunde and Greep, (1936) state that of many substances tried, L.H. is the only one that suppresses the persisting corpora lutea of hypophysectomised rats; Selye, Collip and Thomson (1934) had previously remarked on the fact that hypophysectomy during pregnancy did not affect the corpora lutea of pregnancy, though here the placenta must be taken into account. Leonard, (1938) has demonstrated L.H. in the pituitary of birds which form no corpora lutea. Marrian and Butler (1937) have reviewed the work which deals with activation of P.U. and A.P. by various agents, the stimulating effect of oestrin on the pituitary, and the bearing which such work has on our conception of pituitary activity and the control of the corpus luteum. Their article should be consulted for an indication of the trend of research on the gonadotropic hormones.

It seems prudent to regard the immature mouse or rat merely as a convenient "reagent" for classifying certain gonadotropic principles without generalising as to their function. It is not impossible that the random anomalies just quoted may be explained by the existence of a delicately adjusted proportionality or alternation between the secretion of the two which is characteristically different in different animals, and which is partly controlled by the ovaries, but this cannot be taken for granted. It is therefore necessary to seek for direct evidence that P.U. is qualitatively different from *human* pituitary gonadotropic hormone. Besides the different reactions from human pituitary implantation and P.U. injection described by Philipp (1930 b), Leonard (1933 a) has shown that human A.P. causes the characteristic acceleration of growth in the immature rat ovary beyond that obtained by P.U., that it stimulates the testes of cockerels, and that in terms of rat-units its minimum rabbit-ovulating dose is much less than that of P.U. According to Bokslag (1937), however, the rat ovary does not distinguish between the two readily. Hamburger (1933) also worked exclusively with human material, and although he assumed in most experiments that F.S.U. was the same as A.P., his protocols show that he was justified in describing a difference. The very existence of F.S.U., which is beyond doubt different from P.U., and which presumably must come from the pituitary, shows that whole pituitary secretion cannot be the same as P.U. The occurrence of this characteristic gonadotropic substance in the blood, urine and placenta in pregnancy, its constant association with chorionic tissue, and the simultaneous default of the pituitary, which yields only negative results, must therefore be taken as

strong evidence that placental tissue is the actual site of production. On the other hand, if F.S.U. and P.U. are regarded respectively as the F.S.H. and L.H. of human pituitary, the fact that F.S.H. can be excreted alone raises the possibility that L.H. can in its turn. Only proof of the identity of whole pituitary secretion with F.S.U. could eliminate this final objection. The occasional appearance of a fully positive Zondek-Ascheim test in the absence of chorionic tissue (e.g., Neumann, 1934, in women with signs of masculinity) also serves to keep alive the germ of doubt.

Experiments on lower animals. The gonads and accessory organs undergo atrophic changes on hypophysectomy, yet in the rat (Pencharz and Long, 1931; Selye, Collip and Thomson, 1933 a), cat (Allan and Wiles, 1932) and mouse (Selye, Collip and Thomson, 1933 c) the pituitary is not essential for the maintenance of pregnancy. This suggests that its function is replaced, and the persistence of the corpus luteum of pregnancy (Selye, Collip and Thomson, 1934) is additional evidence for this. Klein (1933) showed that after removal of the foetuses on the 15th day of pregnancy from the rabbit, the endometrium and myometrium were still typical of pregnancy on the 20th day, provided the placentae were left in situ. In careful work on the rat Klein (1935) studied the vaginal epithelium in normal pregnancy, after complete uterine evacuation during pregnancy, after removal of the foetuses and leaving the placentae, and after removing both foetuses and ovaries. So long as placentae and ovaries were present, the vagina remained mucified; in the presence of the ovaries alone it became cornified; after oöphorectomy the placentae were unable to maintain the epithelium in its "pregnant" state, and it showed degeneration. Klein postulated a trophic effect of the placenta on the corpus luteum. Selye, Collip and Thomson, (1935) believe on the basis of the endometrial picture after oöphorectomy, that the rat placenta secretes progestin in addition, but insufficient to make the uterus accommodate itself to the growing foetuses. They present good evidence that the cause of foetal death is lack of resilience of the uterus. The mammary glands were maintained. With the ovaries intact, the placentae after foetal removal were delivered at times not differing widely from normal full term. (See also Selye and McKeown, 1935.) In the pregnant mouse, Newton (1935) found that the foetuses could be destroyed mechanically, without open operation, and that the retained placentae were delivered at normal full term, which was followed by oestrus. This also followed complete earlier uterine evacuation and was therefore actively suppressed in the

presence of the placentae. Loss of the placentae was accompanied by a sharp drop in weight (maintained after foetal destruction) apparently due to an adjustment of the water content of the animals (Brooksby and Newton, 1938). All of these effects are compatible with a humoral effect of the placentae on the ovary, except the evidence for direct secretion of progestin advanced by Selye, Collip and Thomson. They are supported by Haterius (1936), who found that the rat would maintain pregnancy in the absence of the ovaries, provided all fetuses but one were removed, and all the placentae allowed to remain. Courrier and Gros (1936) have found that a carefully timed early oöphorectomy in the 2-week pregnant cat, while causing foetal absorption, does not prevent placental growth for at least a further three weeks, and that hypertrophy of the uterus is maintained.

(*Note added in proof.* Since this was first written, Kirsch, 1938, has confirmed the delivery of placentae at normal full term in the rat after early foetal removal. Newton and Lits, 1938, have found in mice that retained placentae determine the normal reabsorption of the symphysis pubis if the ovaries are also present, but not if they are removed. The mammary glands, on the other hand, tend to develop normally whether the ovaries are present or not. This proves the existence of a double placental influence mediated partly through the ovary and partly directly or through other channels. Astwood, 1938, finds that some extracts of rat placentae act on the ovary, others on the mammary gland. There is no evidence that the maintenance of the mammary glands is due to progesterone.)

General considerations. Since the ovaries can be removed without terminating human pregnancy, any function of the placenta as an ovarian stimulant is not essential in man. Halban (1905) first advanced the view that the ovary, after the early stages of pregnancy, relinquished its functions altogether, and that these were taken over and extended quantitatively by the placenta. In view of the necessity of the ovaries in most animals, it is difficult to believe that the few which can dispense with them, and which otherwise bear no resemblance to each other (e.g., guinea pig, horse, man), are without some source of at least progestin. In man it has been demonstrated that oestrin is produced after oöphorectomy, and it must be admitted that one of the most likely sources of these ovarian hormones is at present the placenta. Clauberg (1936) asks if the ovary is useless, what can be the function of the pituitary, since its only known influence is gonadotropic? The answer is, of course, that it may sustain the extra-ovarian production of oestrin

and progestin. But there is little evidence to associate the large quantities of gonadotropic substance which actually circulate in pregnancy with the pituitary, while it is difficult not to associate them with the placenta. It cannot be denied that, in spite of a natural reluctance to regard a temporary organ like the placenta as a ductless gland which dominates the whole body, one is always on the defensive in pointing out that the evidence is inconclusive.

It is ironical that the one hormone which would seem essential for pregnancy—progrestin—is the least satisfactorily linked with the human placenta, while it is difficult to ascribe a function to the oestrin and gonadotropic hormone which are excreted. Their quantity, measured in terms of activity, is so large and they so saturate the body, that it would be simpler to regard them rather as products of metabolism than hormones if such an explanation did not raise further difficulties. It is possible that the local concentration of oestrin in the placenta is important for the hypertrophy of the uterus, and that the "overflow" is a necessary accompaniment of this. Loewe and Voss (1926) showed that the wall and mucosa of a single horn of the rabbit uterus would grow larger than its fellow on local application of oestrin. The possibility that oestrin may play a part in parturition by increasing the contractile sensitivity of the uterine muscle is treated by Robson (1936) in a review of the whole subject of hormones during pregnancy. Turner, Frank, Lomas and Nibler (1930) in a careful investigation of the excretion of oestrin in pregnant cattle found that dairy cows had a significantly greater output than those which were raised for meat. This points to a relation between the oestrin of pregnancy and the mammary gland, and it is quite reasonable to suppose that at any rate part of the function of the oestrin is to prepare the mammary gland for lactation. That oestrin has no function in pregnancy, and that excretion in an inactive form (oestriol glucuronide) takes place is a possibility, but too little is known about its metabolism to justify more than a mention of this. Coincident with the growing interest in the metabolism of oestrin comes the finding by Venning and Browne that pregnandiol glucuronide is excreted at a time in the menstrual cycle when the corpus luteum is undoubtedly active. The form in which oestrin is excreted is therefore at present no evidence against its exerting functional activity.

The gonadotropic hormone of pregnancy seems to have little action, or a destructive action, on the human ovary (see previous citations), and

it reaches the peak of its excretion at just about the same time as the ovary becomes superfluous. It continues to be excreted throughout pregnancy, and it is possible that it exerts an inhibitory action on the follicular system of the ovary, after having put this out of action by a "mass attack" in the second month. The corpus luteum, which probably checks cyclical changes in the first weeks, would presumably be incapable of doing so later on. This is purely speculative, but seems to have more foundation than Clauberg's suggestion that the gonadotropic hormone is the "Auflockerungkomponent" for the genital tract. Leaving aside the question of chorionepithelioma, in which much lower oestrin values are found than in pregnancy, it is interesting to note that in the toxaemias of late pregnancy, Smith and Smith (1934, 1935 a) report strong evidence that both in the urine and placenta there is excess of gonadotropic hormone and a subnormal amount of oestrin. They tentatively postulate an antagonism between the two.

In conclusion, it can only be said that animal experiments with retained placentae and the correlation of the presence of placental tissue with certain changes and events in man point to the placenta having an endocrine function in controlling gestation. The difficulty is to express this in more precise terms and to prove it in detail, particularly in man. The oestrin and gonadotropic hormone which have to be accounted for in human pregnancy are rather an embarrassment than otherwise. While the controversy as to whether they arise in the placenta is not finally settled, there remains in the background the next question of how they could be linked with any simple view of placental activity, such as Halban's original idea that the placenta takes over the function of the ovary. It almost seems that we must treat the pregnant animal as a different animal, and start again from the beginning in determining hormonic relationships. This may hold for other physiological processes too. A glance through the journals published during the last few years reveals the placenta as a store of practically everything which is sought in it (including the male sex hormone), as well as the site of important enzymic processes. If we are to assume that every particular in which it differs chemically from other tissues denotes a corresponding functional activity, then the placenta, in point of versatility combined with simplicity of structure, easily outdoes the anterior pituitary. In many ways it seems to resemble the liver, and it is doubtful if it will be possible for many years to classify and pigeon-hole it as having a characteristic circumscribed function.

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PHARMACOLOGY OF THE ANESTHETIC GASES

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There appears to be little justification for a review of the narcotic gases which is limited in scope to a discussion of the pharmacological actions of the four agents in common clinical use. The reviewers have, therefore, felt it to be incumbent upon them to attempt a survey of the known gaseous[1] compounds in an effort to determine if possible what might logically be expected from the future search for new anesthetic agents. Certain generalizations regarding the relation between the physical and chemical properties of the gases and their narcotic action are apparently justified, and these have been presented and briefly discussed. No attempt has been made to consider the extensive clinical literature relating to nitrous oxide, ethylene, and acetylene, nor to present an historical background for this discussion. Only those references to fundamental studies have been included which appear to be representative. A considerable amount of space has been devoted to cyclopropane since no previous review of the pharmacological actions of this compound is available.

When an attempt is made to list the qualities which are expected in the "ideal" anesthetic gas, the desires of four individuals must be considered. The *patient* desires not only a rapid and pleasant induction with a gas which is non-irritating and free from unpleasant odor, but also a recovery period which is devoid of discomfort. The *surgeon* would be satisfied if the gas was non-explosive, failed to increase the capillary bleeding, and above all was capable of effecting complete muscular relaxation. The *anesthetist* would expect such a gas to possess a good margin of safety; to be eliminated from the body entirely unchanged; to produce a minimum of functional or organic injury both during and following exposure; and to be sufficiently potent so that a high percentage of oxygen may be administered during anesthesia. Further, the interchange of this gas between the atmosphere and the tissues should

[1] All compounds with a boiling point below 20°C. at 760 mm. Hg pressure have been arbitrarily considered as gases.

be sufficiently rapid to allow "moment to moment" control of the depth of anesthesia. The *manufacturer* would consider the ideal anesthetic gas to be one which can be produced simply and inexpensively, is easily purified, and remains chemically unchanged during storage.

There is no known gas which meets all the requirements specified above. Except for its lack of anesthetic potency, nitrous oxide would qualify for the ideal narcotic gas. It would therefore appear logical to search for new gases which are related chemically to this substance. Unfortunately, the chemist offers little encouragement regarding the preparation of new gaseous compounds of nitrogen, and those in existence are either too toxic or too irritating. The gaseous elements do not possess narcotic properties, and the gaseous compounds of the elements, other than carbon, have been found to be of little value because of their toxicity. Although the hydrocarbons possess the desired anesthetic strength, their use is accompanied by the hazard of fire and explosion. The hydrocarbon series appears at present to be the only available source of new gaseous agents. Before turning to a discussion of this group of organic compounds, nitrous oxide will be considered, it being the only inorganic gas known at the present time to possess useful narcotic properties.

Nitrous oxide (N_2O) Mol. Wt. 44; B. P. −89°C.; Sp. Gr. 1.53 (air = 1). A colorless, odorless gas with a slightly sweet taste. This compound is non-inflammable but will support combustion even in the absence of oxygen. At high pressures, nitrous oxide-oxygen mixtures are explosive in the presence of oil (8). Blomfield (4) lists the contaminants which must be removed before the gas may be used clinically. The solubility coefficients,[2] at 37.5°C. are: water, 0.440 (109); blood, 0.470 (109); oil, 1.40 (73). These values give an oil-water coefficient of 3.2. The lack of agreement regarding the solubilities of this gas is in part due to the want of accurate methods of analysis. The solubility in blood undoubtedly varies with changes in cell volume, water and lipoid content, since Nicloux (78) and Siebeck (109) found that cells absorb more nitrous oxide than plasma. Siebeck also obtained solubility coefficients for plasma which are lower than those for water.

Several early investigators (59) obtained what they thought was a characteristic spectrum with blood containing nitrous oxide. This was interpreted to mean that nitrous oxide forms a loose combination with hemoglobin. Although this evidence is not very convincing, in view

[2] Throughout this paper, solubilities are expressed in terms of λ, the Ostwald solubility expression.

of the small difference in solubility between water and blood, Killian and Moritz (56), who have studied this problem more recently, have not been able to conclusively disprove the existence of such a combination. They found the absorption bands in the presence of nitrous oxide to be slightly different than those of oxyhemoglobin.

The diffusion rate of nitrous oxide is quite rapid, judging by the short induction and recovery time. The gas disappears within a relatively short time after injection into the subarachnoid space (1), and into the peritoneal or pleural cavities (27, 113). Coryllos and Birnbaum (13) studied the rate of diffusion from the lung into the blood by filling an isolated lobe with various gases. They then determined, by several different methods, the rate of deflation of the experimental lobe, while the remaining lung tissue was ventilated by artificial respiration. The average time required for complete disappearance is: nitrous oxide, 17 to 35 minutes; ethylene, 13 to 29 minutes; oxygen, 15 minutes; carbon dioxide, 4 minutes; and nitrogen, 16 hours. Similar observations have been made by others (99). Further evidence of its rapid diffusion is found in the fact that an appreciable amount of this gas is lost through the skin during anesthesia (83).

Several investigators have determined the amount of nitrous oxide in blood during anesthesia (32, 33, 49, 52, 78, 80). Jolyet and Blanche (49) found 29 volumes per cent nitrous oxide in blood after allowing animals to breathe 62 per cent nitrous oxide for seven and one-half minutes. This represents complete saturation when compared with the solubility coefficient for blood quoted above. Gréhant (33) obtained only 26 volumes per cent in blood after 15 minutes of anesthesia with 75 per cent nitrous oxide; i.e., 74 per cent saturation. Greene et al. (32) analyzed the arterial blood of dogs after 3 to 10 minutes of anesthesia. They found between 18.9 and 26.8 volumes per cent nitrous oxide, when the animals were breathing 85 to 96 per cent concentrations of this gas. The average value, 23 volumes per cent, represented approximately half saturation. Although these latter experiments appear to be the most extensive of any reported, it is nevertheless difficult to draw definite conclusions concerning their value since no statements are made as to time of sampling, anoxia was uniformly present, and their method of analysis for nitrous oxide is open to criticism. A few observations by Gréhant (33) and Nicloux (78) indicate that a major portion of the nitrous oxide is eliminated from the blood in a few minutes. Nicloux could detect no nitrous oxide in either arterial or venous blood five minutes after anesthesia.

There is no evidence that this gas is altered in the body, although the idea has been entertained by some anesthetists that the oxygen atom in the nitrous oxide molecule is available to carry on body oxidation. This concept may have originated in the work of Olivier and Garrett (80) who found 11.23 volumes per cent of *nitrogen* in dog's blood after nitrous oxide anesthesia, presumably a residue from nitrous oxide destruction. Although this value represents an impossible supersaturation, it is peculiar that these investigators obtained control values of 1.8 volumes per cent nitrogen, which would be considered fairly accurate today. The results thus far reported, relative to the absorption and elimination of this gas are so discordant that the reviewers are hesitant to accept any one as representing the facts, and believe that the whole problem needs reinvestigation, since more accurate methods of analysis are now available (81, 84), and the principles governing body saturation and desaturation with the gaseous agents have been more clearly elucidated (44).

Nitrous oxide has a very low anesthetic potency. It is generally agreed that satisfactory anesthesia, according to present day standards, cannot be obtained in laboratory animals without simultaneous anoxia. Luckhardt and Carter (67) were unable to obtain anesthesia in the mouse, rat, guinea pig, rabbit or cat with 85 per cent nitrous oxide. Ninety-five per cent was required to produce anesthesia in the mouse and rat although they state that the same concentration of nitrogen or hydrogen was almost equally effective. The larger animals were considered to be anesthetized with 90 per cent nitrous oxide after 20 to 30 minutes' exposure. Greene and Currey (31) found it necessary to reduce the oxygen concentration in nitrous oxide-oxygen mixtures to an average of 7.6 per cent for light, and 3.4 per cent for deep anesthesia in the dog. Leake and Hertzman (62), among other investigators, conclude that it is impossible to obtain true anesthesia in the dog without some degree of anoxemia.

The contention of Paul Bert (3) that anesthesia could be obtained with nitrous oxide without anoxia, if the partial pressure of the gas was raised to one and one-quarter atmospheres, is placed in question by the experiments of Brown, Lucas and Henderson (8). They could not obtain surgical anesthesia in the rabbit or cat at nearly two atmospheres of nitrous oxide, if the partial pressure of oxygen was equal to that in the atmosphere, 156 mm. Lendle (64) obtained what he considered to be anesthesia, in the mouse, at a partial pressure of nitrous oxide of 1440 mm. In these experiments the partial pressure of oxygen

was above 150 mm. He found that nitrous oxide at a partial pressure of 2440 mm. is lethal for this animal, even though the partial pressure of oxygen was never below 186 mm. in any of his experiments at these high pressures. The studies of Graham (30) indicate that nitrous oxide under pressure (3 to 4.3 atmospheres) produces a reversible narcosis in isolated muscle although lower concentrations, or nitrogen at 10 atmospheres pressure, were ineffective. This problem does not appear to be entirely settled and the reviewers believe that similar experiments should be repeated on some larger animal, such as the dog, or man.

It is virtually impossible to state that a certain concentration of this gas will accomplish a stated degree of anesthesia in any animal or human individual. Neither can it be said that every nitrous oxide anesthesia on man is accompanied by anoxemia. Satisfactory anesthesia may be obtained in certain individuals of a low reflex excitability, such as the aged, even though 20 per cent or more oxygen is allowed. In most subjects, however, it is just possible to enter the first plane of surgical anesthesia with 90 per cent nitrous oxide and 10 per cent oxygen (36). The data obtained by Davidson (15), relating certain signs of depression to definite concentrations of this gas, have been often quoted, but as these values were all obtained on one individual, they can hardly be considered representative. The level at which consciousness is lost shows a range of individual variation from 35 to 70 per cent (104). Subanesthetic concentrations are definitely analgesic and find a place of usefulness in clinical practice (104).

The presence of small quantities of nitrogen in the lung and tissues interferes with the attainment of the greatest possible partial pressure of nitrous oxide and thus delays the advent of maximum anesthesia. McKesson (71) developed a technique which he termed "secondary saturation," the success of which was explained primarily on the basis of the elimination of the remaining nitrogen from the body. After several minutes of anesthesia with a nitrous oxide-oxygen mixture, he allows the patient to inhale pure nitrous oxide until the pupil dilates, tonic muscular spasm or rigidity develops and respiration becomes slow or ceases entirely. One breath of pure oxygen is then administered and the original anesthetic mixture again established. There follows a period in which complete muscular relaxation is obtained; in fact it may last throughout the operation, if the oxygen concentration is not raised too high. The efficacy of this procedure is definitely established. It appears, however, that a more probable explanation of this profound anesthesia lies in the depression following the acute anoxia, although it

is not clear why the musculature should remain relaxed for such a long period. It would be of interest to determine experimentally whether the paresis of the muscle is of central or peripheral origin. In the hands of the expert, this procedure is relatively safe because of the tolerance of the healthy organism to short periods of anoxia.

If the partial pressure of oxygen is reduced to 70 mm. or below, anoxia contributes its share to produce a deeper anesthesia (2, 8, 64). Prolonged exposure to a severe grade of anoxia (5–7 per cent) is much more undesirable than the procedure of McKesson since it is fraught with the danger of producing permanent damage to the parenchyma of the brain (14). Lowenberg et al. (65) contend that the cerebral pathology observed is not due to anoxia but to a specific action of nitrous oxide. They have little support for this view.

The direct effects of nitrous oxide on respiration, the circulation, and other vital functions, are of little significance. If the partial pressure of oxygen is maintained at 21 per cent, respirations may become slightly slower and deeper, with little change in minute volume. The circulation is only slightly influenced, the major effect being a dilatation of the peripheral capillaries. This is accentuated by anoxia (29). The degree of muscular relaxation obtained with nitrous oxide is what might be expected from the elimination of cerebral influences (24).

If the concentration of nitrous oxide is raised, so that the partial pressure of oxygen is diminished, then certain signs are seen which are ordinarily stated to be characteristic of nitrous oxide anesthesia. They are, for the most part, due to the accompanying anoxia. The rate and depth of respiration is increased and blood pressure becomes elevated, until the anoxia reaches a critical level. Wright and Thompson (121) studied the blood pressure on normal subjects when breathing pure nitrous oxide and obtained an initial psychic rise which was in most cases followed by a gradual or precipitous fall, undoubtedly due to the paralyzing effect of anoxia. Part of the rise in blood pressure may be due to pressor reflexes which arise from sensory stimulation accompanying the operative procedure under such light anesthesia.

Downs (21) has shown that nitrous oxide does not prevent, and may increase, the reflexogenic activity of the carotid sinus, although the evidence for the latter is not clear-cut since anoxia was not rigidly excluded in these experiments.

A fall in the alkali reserve and pH (62), and a rise in blood sugar and lactic acid (7), occur if anoxia is present. Miller (75) noted a marked increase in the contractions of the stomach, ileum, and colon of the dog

during anesthesia. These effects were probably also due to anoxia, although no mention is made in his paper of gas concentrations.

The utility of nitrous oxide as an anesthetic agent is greatly enhanced if it is used in conjunction with other depressant drugs. In fact, if adequate premedication with morphine-scopolamine, a barbiturate, tribromethanol, or other non-volatile agents is obtained, prior to the use of nitrous oxide, a fair percentage of major surgery can be accomplished. Such effects are, however, only additive and it may not be expected that satisfactory abdominal relaxation can be obtained in most instances without such medication.

The reviewers are impressed with the lack of accurate quantitative data concerning nearly every phase of nitrous oxide anesthesia. This may be due to the fact that the anesthetic potency of nitrous oxide is low and that any individual subject, or animal, may have such a high level of nervous excitability that anesthesia is impossible with this agent. Further, its peculiar chemical properties render it difficult to estimate quantitatively, and its study in recent years has been neglected for the more promising compounds. This gas is, however, of too great practical value, if properly used, to be discarded, and deserves further detailed and careful study.

The saturated hydrocarbons (C_nH_{2n+2})

	MOL. WT.	B. P.	SP. GR.	ANESTHETIC RANGE
		°C.	*air = 1*	*vols. per cent*
Methane—CH_4	16	−160	0.55	87
Ethane—$CH_3 \cdot CH_3$	30	−85	1.07	
Propane—$CH_3 \cdot CH_2 \cdot CH_3$	44	−37	1.53	93
Isobutane—$(CH_3)_3CH$	58	−10	2.01	45–55
n-Butane—$CH_3 \cdot (CH_2)_2CH_3$	58	−1	2.01	22–25
Neopentane—$(CH_3)_4C$	72	+9	2.50	27–34
Ethyl chloride—$CH_3 \cdot CH_2Cl$	64.5	+12	2.23	5–8

The explosive range of most of these compounds may be found in Killian's monograph (53). All of the gaseous members of the homologous series of straight chain saturated hydrocarbons and their isomers have been examined for their anesthetic properties.

Methane. Richardson (92), in 1867, studied the anesthetic properties of methane and considered it a good anesthetic. Regnault and Villejean (89) found that 83 per cent methane in oxygen would not induce anesthesia. Experiments at increased pressures indicated that a par-

tial pressure of methane of 760 mm. would not effect satisfactory narcosis if adequate oxygen was allowed. Herter (45) failed to obtain anesthesia with 79 per cent methane. Brown (6) found that an 87 per cent concentration was necessary to abolish the response to electrical stimulation in the cat, while the continued inhalation of 90 per cent gradually paralyzed respiration.

Ethane. No recent studies of ethane have been made. Gwathmey (37) records that Richardson studied ethane and compared its action to that of pentane. There is little reason to believe that its narcotic action would be materially different from methane or propane.

Propane. Brown and Henderson (10) found propane to offer little promise as an anesthetic. Concentrations as high as 93 per cent would not effectively abolish the response to electrical stimulation although 82 per cent lowered the blood pressure of the cat. Concentrations as low as 50 per cent caused respiratory arrest for one or two minutes. The circulation is seriously impaired when 87 per cent propane is exhibited.

In a recent study, Stoughton and Lamson (111) have investigated the anesthetic properties of the butanes and pentanes. This had not been done previously. None of the group is promising. They obtained the following results with the gaseous members.

n-Butane. A concentration of 20 to 25 per cent butane is necessary to produce relaxation in the dog but death occurs after a short period of exposure.

Isobutane. Anesthesia is induced with 45 per cent, whereas a concentration of 55 per cent is lethal.

Neopentane. This compound differs from the other agents in that a greater degree of reflex excitability is noted in mice both during induction and anesthesia.

Halogen derivatives. The gaseous halogen derivatives of this series, such as methyl chloride, are with one exception, too toxic to be of value as anesthetics (115). It seems to the reviewers that the halogenated ethane, ethyl chloride, should logically be included in this discussion since it is a gas at ordinary temperatures and pressures. This compound, however, is usually classified with the volatile group of anesthetics and limitations of space prevent its consideration. Kochmann (58) gives a good review of its pharmacological actions. Several other references (23, 35, 40, 115) are appended which give summaries of its actions.

Although no extensive and carefully controlled study has been made of this homologous series, and accurate data concerning solubilities, etc.,

are not available, the evidence at present points to the validity of the generalization of Brown and Henderson who stated in 1926, "the methane series will probably not yield an anesthetic gas."

The olefines (C_nH_{2n})

	MOL. WT.	B. P.	SP. GR.	ANESTHETIC RANGE
		°C.	*air = 1*	*vols. per cent*
Ethylene—$CH_2:CH_2$	28	−103	0.96	80–140
Propylene—$CH_2:CHCH_3$	42	−47	1.46	40–65
α-Butylene—$CH_3CH_2CH:CH_2$	56	−5	1.94	20–30
β-Butylene—$CH_3CH:CHCH_3$	56	+1	1.94	15–25
γ-Butylene—$CH_2:C(CH_3)_2$	56	−6	1.94	30–45
Vinyl chloride—$CH_2:CHCl$	62.5	−13.9	2.17	7–11

The anesthetic properties of all of the gases, and many of the low boiling point liquids (the amylenes, etc.), of this unsaturated homologous series have been investigated. In contrast to the saturated hydrocarbons, these compounds are quite reactive chemically. There is a tendency for the more complex members to polymerize even on storage. Only one of this group, ethylene, has been found satisfactory for anesthesia in man.

Ethylene. A colorless gas with a characteristic odor and taste, the latter properties rendering the agent unpleasant to some individuals (68). The gas may be stored without undergoing chemical alteration. The most likely impurities are carbon monoxide, carbon dioxide, acetylene, aldehydes, hydrogen sulphide and phosphine. The gas as marketed for anesthesia is, however, free from these impurities. The explosive range in air is 3 to 28.6 per cent; in oxygen 3.1 to 79.9 per cent (50). The solubilities of the pure gas are:

TEMPERATURE	WATER	BLOOD	OIL
°C.	λ	λ	λ
20	0.124 (54)	0.130 defibrinated ox (54)	1.595 sesame (54)
37.5	0.09 (34, 70)	0.140 human (34, 70)	1.30 olive (74)

The oil-water coefficient is about 13. As Grollman (34) has pointed out, the solubility in blood varies with its water and lipoid content so that no absolute value can be given for this ratio. The statement of Grollman is substantiated by the observations of Nicloux and

Yovanovitch (79) who found that 70 to 80 per cent of the ethylene present in whole blood is in the cellular elements and the remainder in the plasma. The same workers found the distribution coefficient $\left(\frac{\text{blood concentration}}{\text{air concentration}}\right)$ *in vivo* to be 0.12 to 0.15. These values are of the same order as would be expected from the absorption coefficients obtained *in vitro*.

Several investigators state that the rate of saturation and desaturation of the blood with ethylene is very rapid. Nicloux and Yovanovitch could detect no ethylene in the arterial blood two minutes after administration was discontinued. It is probable that their analytical methods were not sufficiently sensitive to determine small quantities of the gas. While their finding is in harmony with the rapid recovery from anesthesia, it must be remembered that a considerable quantity of such a weak agent as ethylene may be present in blood and tissues without the accompaniment of anesthesia. The tissues are known to retain appreciable quantities of the gas for hours following administration (105).

Ethylene produces little or no irritation of mucous membranes or of the pulmonary epithelium (13). Inhalation of the pure gas may evoke reflex choking or swallowing and may lead to a sense of suffocation. Induction is usually smooth and an excitement stage is seldom present (68). Usually salivation is minimal and mucous secretion is not appreciably increased (68, 97). Concentrations of ethylene between 80 and 90 per cent[3] are required to anesthetize the common laboratory animals including mice (74), rats (94, 67), guinea pigs (67, 94), rabbits (67), cats (9, 20, 67), and dogs (5, 62, 67). Halsey, Reynolds and Prout (38) found the ratio $\left(\frac{\text{maximal non-lethal dose}}{\text{minimum narcotic dose}}\right)$ for mice to be 1.65. This was obtained by raising the partial pressure of ethylene to 1.4 atmospheres, at which level death occurred. Leake and Hertzman (62) found 85 per cent ethylene effective for anesthesia in the dog but no surgical procedures were attempted in their experiments. The reviewers and others (116) have not been able to obtain satisfactory surgical anesthesia in the dog without previous medication.

The anesthetic range for man varies quite widely with the individual. Twenty-five to thirty-five per cent ethylene produces analgesia (104).

[3] Many statements regarding the effects of specific concentrations of ethylene, as well as the other gases, are based upon readings taken from gas machines, rather than gas analysis, and may therefore be inaccurate.

This range of concentration is usually not sufficient to render the subject uncoöperative, and consciousness is usually retained (104). An occasional individual will be found who loses consciousness when breathing 40 per cent ethylene, but 50 to 60 per cent is ordinarily required. In the experiments of Davidson (17), 61 per cent induced unconsciousness within five minutes. Eighty to eight-five per cent ethylene is usually required to effect first plane anesthesia; that is, it will obliterate the lid reflex and carry the patient past the level of reflex swallowing or vomiting (36). If the concentration is increased to 90 per cent, the extra-ocular muscles may be almost, but not quite, relaxed (36). It is not to the best interests of the subject to use concentrations of ethylene greater than 90 per cent for prolonged anesthesia since anoxia becomes a contributing factor. Ethylene, like nitrous oxide, is unsuited for anesthesia if complete muscular relaxation is needed to accomplish a given surgical performance. The deepest ethylene anesthesia will not eliminate the reflex laryngospasm which is initiated by the stimulation of sensitive regions such as periosteum, peritoneum, or direct trauma to major nerve trunks.

Respiration during first plane anesthesia is regular (36, 68). The rate and depth is usually increased, but may be decreased, particularly after premedication. The sensitivity of the respiratory center to carbon dioxide remains essentially unchanged (57). It is difficult to differentiate between the direct respiratory effects of ethylene and those which occur from the reduction of oxygen, since some degree of anoxia is an almost invariable accompaniment of anesthesia with this gas, as with nitrous oxide. Since mild degrees of anoxia cause an increase in minute volume respiration, it becomes difficult to ascribe the increase in respiration to a direct action of the gas. It is impossible to effect respiratory arrest by the action of this gas alone. If it occurs, it results from the combined depressant action of the drug and anoxia.

An elevation of blood pressure is commonly seen in the cat (24), dog (67), and man (36). It probably occurs as a result of the accompanying splanchnic constriction. Like the effects on respiration, it is difficult to prove this a direct action of the gas, because of the complication of anoxia. Bouckaert (5) found 90 per cent ethylene to exert little effect on the isolated rabbit heart. Caine and Reynolds (12) and Killian (54) report that no evidence of disturbance in cardiac automaticity or conduction is obtained by the electrocardiograph. Kurtz, Bennett and Shapiro (60) observed less cardiac arhythmia with ethylene than with any other agent except procaine. Franken (24) noted that ethy-

lene produced an increase in the circulation and cardiac output of patients.

The tone of the smooth muscle of the stomach, colon, and the intestine of the dog is increased, according to the studies of Miller (75). Johnston and Ivy (48) noted a prolongation of the emptying time of the human stomach during narcosis, due either to a diminished tone of the musculature or to a pylorospasm. They found gastric secretion to be diminished during ethylene anesthesia.

Leake and Hertzman (62) state that the pH and carbon dioxide content of arterial blood of the dog tends to fall slightly, but not beyond normal limits, following one-half hour of anesthesia with 85 per cent ethylene. If anoxemia is present, an initial increase in the pH occurs accompanied by inconstant changes in the carbon dioxide content. This alkalosis persists for 15 to 30 minutes and is followed by a fall in pH and carbon dioxide content. The increase in respiratory activity from anoxia probably explains these results since they are similar to those which occur following hyperventilation initiated in other ways. Leake (61) found a slight increase in the acetone bodies of the blood, but this change was insignificant when compared with the change produced by ether.

Walton (116) states that ethylene does not appreciably diminish the twenty-four hour urinary output of normal dogs nor affect significantly the chloride, urea, or phosphorus output, or dye excretion.

The influence of ethylene on the resistance of erythrocytes to the hemolytic action of hypotonic saline is insignificant (63). According to deGouweia (20), coagulation time is increased, but the increase in capillary bleeding which occurs during ethylene anesthesia is probably due to the relaxation of the peripheral capillaries by the direct action of the gas and anoxia.

A number of investigators have administered ethylene repeatedly over long periods in order to study the effects of the gas on body tissues by histological examination (5, 25, 91). None of these studies indicate more than a slight amount of congestion or fatty change in the parenchymatous tissues.

The consensus among investigators is, that in the absence of anoxia, the effects of ethylene on general metabolic activity are of little significance.

Propylene. A colorless gas with an odor similar to that of ethylene or acetylene. Although it is stated that this gas is quite stable, its puri-

fication is difficult, and the lack of uniform results reported in the literature relative to effective concentrations may be partly explained by the presence or absence of higher boiling point fractions (38). Henderson and Smith (43) have recently shown that hexanes and hexenes may be contaminants. The explosive range in air is from 2 to 7 per cent; in oxygen, 3 to 45 per cent (53). The solubility coefficients (54) at 20°C. are: water, 0.136; defibrinated ox blood, 0.221; sesame oil, 7.217. The oil-water coefficient is 52.

Meyer and Hopff (74) reported in 1923 that 50 per cent propylene would narcotize mice. This paper was followed shortly by several reports of more extensive studies. Halsey, Reynolds and Prout (38) found the ratio $\left(\frac{\text{maximal non-lethal dose}}{\text{minimal narcotic dose}}\right)$ for the mouse to be between 1.66 and 1.85, depending upon the purity of the gas used. The factor of safety was thus almost identical with that of ethylene. The narcotic action of the gas on all of the common laboratory animals has been studied: mice (38, 54, 74), rats (93), guinea pigs (95), rabbits (54, 95), cats (10, 38, 95), dogs (38, 95). It has been found that all species are narcotized with concentrations between 35 and 55 per cent. Concentrations between 55 and 65 per cent are lethal to small animals such as the mouse and the rat, and produce evidence of cardiovascular injury in the larger species (cats and dogs), although 70 to 80 per cent is usually required to kill the latter. According to Riggs and Goulden (95), respiration fails before the circulation, but Halsey et al. (38) state that in most instances they fail simultaneously. Although 40 to 50 per cent propylene induces narcosis in the cat and dog, it does not abolish completely the reflex response to sensory stimulation (38). It can not be said that the gas produces good anesthesia even in concentrations which are nearly lethal, since muscular rigidity usually persists throughout (38, 95, 119). Riggs and Goulden found that these signs of hyper-irritability occurred more often with tanked, than with the freshly prepared gas, and the incidence was high with gas from the last part of the cylinder.

A few studies have been made of the effects of propylene on man. Davidson (18) found that 15 per cent induced unconsciousness in 30 minutes, and 24 per cent in 3 minutes. Halsey et al. (38) inhaled 35 and 40 per cent propylene and unconsciousness occurred within 20 seconds. Persistent vomiting followed 6 minutes of inhalation of a 40 per cent mixture. Brown and Henderson (10) found that normal sub-

jects were deeply anesthetized with 50 per cent propylene in oxygen. Riggs reports nine experiments on normal subjects with no undesirable after effects.

An exaggeration of respiratory activity is characteristic with nearly all concentrations in the dog (38). Halsey et al. found a low carbon dioxide content of tracheal samples. Brown and Henderson (10) state that 60 per cent propylene does not have a toxic action on either respiration or the circulation of the cat. They found a slight lowering of the alkali reserve and the pH of blood after one hour of anesthesia. Blood sugar was affected very little. The majority of work indicates that high concentrations, 70 to 80 per cent, exert a toxic action on the cardiovascular system. There is a rapid pulse, a marked fall in blood pressure and the rapid appearance of cardiac arhythmia at this level. Caine and Reynolds (12) found that 25 to 50 per cent induced extrasystoles and ventricular tachycardia in cats and dogs. Similar results were obtained by the authors (119). Killian (54), on the other hand, obtained no fall in blood pressure or arhythmia in the rabbit with 80 per cent propylene. Riggs and Goulden (95) and Kahn and Riggs (51) noted only a sinus arhythmia in their clinical experiments. Reynolds (91), who studied the effects of the gas on isolated tissues, could obtain no evidence of functional injury to the frog heart, the rabbit intestine, or the uterus of the guinea pig. He also exposed mice to the action of 35 per cent propylene, twenty times in 58 days, and obtained little evidence of chronic injury, a moderate amount of fatty change in the liver being the only consistent finding.

Athough at present this gas does not offer much promise of being suitable for clinical anesthesia, it is probable that impurities in the preparations which have been used by some, or all, investigators have contributed an injurious action. It appears that if propylene in pure form can be obtained, further studies of this compound would be desirable.

α-Butylene (ethyl ethylene). Brown and Henderson (10) and Riggs (93) have reported studies on this compound. There is considerable doubt as to the purity of their preparations. Riggs thought that he was working with a mixture which also contained normal butylene. He found that rats were narcotized with a 20 per cent concentration of the gas and died from respiratory paralysis if exposed to 40 per cent. The first named investigators obtained narcosis in the cat with 30 per cent but it was accompanied by muscle rigidity. A fall in blood pressure occurred with higher concentrations.

β-Butylene (*normal*). This gas possesses an extremely unpleasant odor. Schmidt and Shaumann (102) and Killian (54) have studied this gas but admit that it probably was impure and contained some ethyl ethylene. The solubility coefficients (54) at 20°C. are: water, 0.173; defibrinated ox blood, 0.448; sesame oil, 32.45. The oil-water coefficient is 188. The explosive range of all the butylenes is the same: in air, 2 to 16 per cent; in oxygen, 3 to 35 per cent. A concentration of 30 per cent is necessary to induce deep anesthesia in the mouse. This concentration causes a moderate and 50 per cent a severe fall in blood pressure in the rabbit; whereas 70 per cent is rapidly lethal, a complete dissociation of cardiac automaticity occurring just prior to death. In all concentrations, muscle rigidity and hyperventilation were noted.

γ-Butylene (*isobutylene*). Isobutylene has an unpleasant odor and a sour after taste. It is difficult and costly to prepare. The solubility coefficients (53) at 20°C. are: water, 0.120; defibrinated ox blood, 0.249; sesame oil, 17.31. The oil-water coefficient is 144. Killian (54) found that 50 per cent concentration produced good anesthesia in the mouse without hyperirritability. On the rabbit, however, 20 per cent produced hyperventilation, a marked drop in blood pressure, and tachycardia with bigeminus.

Vinyl chloride. Schmidt and Shaumann (102) studied this gas in 1929 and make the following statements concerning its actions. The pure gas has an odor like ethyl chloride, although in narcotic concentrations (7 to 10 per cent) it is practically odorless. Four to twenty per cent mixtures of the gas in air burn with difficulty, and the danger of explosion is less than with any other narcotic hydrocarbon. Rapid recovery occurs in the dog following one hour exposure to five per cent, a concentration which is sufficient to abolish the corneal reflex. Four hours of anesthesia with 10 per cent vinyl chloride results in respiratory arrest, the usual cause of death. A rise in blood pressure occurs with 12 per cent although no evidence of circulatory injury was noted. Histological studies of the tissues of mice and rats exposed to 5 per cent vinyl chloride, four hours daily for five days, failed to reveal any significant pathological change. Patty, Yant and Waite (85), however, noted pulmonary edema and congestion of the liver and kidneys of guinea pigs after exposure to this gas.

Peoples and Leake (86) studied the gas and found the minimal anesthetic range on mice exposed for ten minutes to be 3.5 to 5 millimols (7.8 to 11.2 per cent) in air while its minimal lethal range was 10 to 12 millimols (22.4 to 27 per cent). Their description of the action of the

gas on rabbits and dogs is similar to that already cited. Although this gas has a tendency to polymerize, the latter authors state that it is not great if the gas is stored in the dark.

Dublin and Vane (22) state that dizziness and disorientation occur in man after 3 minutes' inhalation of 2.5 volumes per cent vinyl chloride in air. Recovery was immediate and complete, except for slight headache lasting 30 minutes.

Of the olefines, only ethylene has proven worthy of clinical use. It does not appear probable that the others mentioned will be of value although no final conclusion would be justified.

The alicyclic hydrocarbons (C_nH_{2n})

	MOL. WT.	B. P.	SP. GR.	ANESTHETIC RANGE
		°C.	*air = 1*	*vols. per cent*
Cyclopropane—$\overline{CH_2 \cdot CH_2 \cdot CH_2}$	42	−34.4	1.46	20–35
1.Methyl-Cyclopropane—$CH_3\overline{CH \cdot CH_2 \cdot CH_2}$	56	+5	1.94	15
Cyclobutane—$\overline{CH_2 \cdot CH_2 \cdot CH_2 \cdot CH_2}$	56	−15	1.94	
Cyclobutylene—$\overline{CH : CH \cdot CH_2 \cdot CH_2}$	54	+2	1.87	

Included in this group are certain cyclic hydrocarbons having the same composition as the homologues of ethylene. Cyclobutane has not been studied pharmacologically but appears to be worthy of investigation. Although cyclobutylene appears to be less promising, its investigation should be of interest since it contains a double bond in the ring.

Cyclopropane (trimethylene). The simplest member, cyclopropane was introduced for clinical trial in 1929 by Lucas and Henderson (41, 66) after a preliminary pharmacological study. A preliminary report on 447 clinical cases was published by Stiles, Neff, Rovenstine and Waters in 1934 (110). A complete bibliography to date would include about 100 references.

Cyclopropane is a colorless gas with a characteristic, although not an unpleasant odor. So far as is known, this compound is chemically stable during storage and may be satisfactorily purified. The most likely impurities are propane, propylene, higher hydrocarbons, ethers, alcohols, alkyl halides, and nitrogen. The gas is inflammable and ex-

plosive. The explosive range in air is 3 to 8.5 per cent; in oxygen 2.5 to 50 per cent (11).

The solubilities of the pure gas as determined by saturation (59), extraction (82), and iodine pentoxide (96) are:

TEMPERATURE	WATER		BLOOD		OIL
°C.	λ		λ		λ
20	0.315 (96)	0.296 (59)	0.680* (96)	0.503† (59)	11.140¶ (59)
25	0.281 (96)	0.278 (82)			9.790‖ (82)
37.5		0.204 (82)		0.457‡ (82)	6.990‖ (82)
39		0.181 (96)		0.451‡ (96)	

* Dog oxalated.
† Ox defibrinated.
‡ Human oxalated.
¶ Sesame.
‖ Olive.

The oil-water coefficient at 37.5°C. is 34.3. The solubility in cod liver and paraffin oils is similar to that in olive oil (82).

The solubility in blood varies with changes in cell volume as well as with the lipoid content of the blood, and therefore is increased after a fat meal (96). Blood cells absorb about two and one-half times as much cyclopropane as blood plasma (96). Robbins (96) found the distribution coefficient $\left(\frac{\text{blood concentration}}{\text{air concentration}}\right)$ in the dog to be 0.492 *in vivo*, and 0.513 *in vitro* at 38°C.; a good agreement. Robbins' determinations indicate that the concentration of cyclopropane in the right heart blood is practically equal to that in arterial blood after 15 minutes of anesthesia. It might be inferred that complete body saturation had occurred at this time. That such is not the case is indicated by the findings of Seevers et al. (105), who noted that several hours must elapse before saturation or desaturation is complete in the dog and rabbit, as indicated by the gas depot method. Similar findings have been obtained on man by the same method (107), and are in agreement with the clinical observation, that appreciable quantities of the gas are lost to the tissues throughout an ordinary anesthesia (117). When the pressure gradients are diminished as saturation or desaturation is approached, the diffusion rate is much prolonged. This is indicated from data obtained by Robbins for the rate of elimination after 2 to 3

hours of anesthesia with 25 to 30 per cent cyclopropane. The venous blood still retained some cyclopropane two hours after anesthesia was terminated. The rate of diffusion of cyclopropane appears to be about twice that of ethylene (105). The great disproportion in potency between these two gases leads to the inference that ethylene is eliminated more rapidly than cyclopropane since induction and recovery are slower with the latter agent. There is no evidence that cyclopropane undergoes any chemical alteration in the body. It does not isomerize to propylene (105).

TABLE 1

Average anesthetic concentrations of cyclopropane as determined by gas analysis

STAGES OF ANESTHESIA (36)	III_1	III_2	III_{3-4}	IV
CHARACTERISTIC SIGN	Man—Roving eyeball Dog—Corneal reflex	Man—Fixed eyeball Dog—Wink reflex	Intercostal muscle paralysis	Respiratory arrest
Cyclopropane, volumes per cent in inspired atmosphere				
After premedication*				
46 men (118)	7.4	13.1	23.3	42.9
4 dogs (106)	5.8	12.7	24.0	31.0
No premedication				
19 dogs (106)	18.8	24.7	33.4	39.4
21 dogs (96)	18.0	27.3	33.6	35.8
17 dogs (72)	18–22		28–34	
Cyclopropane, mgm. per 100 cc. of blood				
21 dogs (96)	13.9	20.6	25.5	28.2

* Ordinary laboratory and clinical doses of morphine.

Cyclopropane is practically non-irritating in anesthetic concentrations. The irritation from higher concentrations (over 50 per cent) is sufficient to initiate a reflex laryngospasm. Absence of irritation and rapidity of absorption allow a smooth induction, usually without excitement, both in animals and man. Passage through the first two stages is usually very rapid and the ordinary signs are missing. This gas possesses analgesic properties in subanesthetic concentrations (3 to 5 per cent) if continuously inhaled (104). Most normal individuals will lose consciousness following the prolonged inhalation of 6 per cent or less. The effective concentrations vary widely with the individual.

The average concentrations required to produce different levels of surgical anesthesia are presented in table 1. The values obtained by different investigators are remarkably uniform. Premedication with ordinary doses of morphine reduces by 10 to 12 per cent the amount of cyclopropane required to effect a given level of anesthesia.

Muscular relaxation sufficient to allow the performance of most surgical procedures may be accomplished with cyclopropane. This gas is not as effective as ether or other volatile agents in this respect, and third plane anesthesia may be required to effect complete abdominal relaxation. In the dog, abdominal muscle relaxation (96) appears to be obtained at concentrations of 22 to 24 per cent. Following premedication, lower concentrations are usually ample for abdominal surgery in man.

In clinical anesthesia the amount of salivary and other secretion from cyclopropane is relatively slight (118). Robbins noted a 250 per cent increase in salivary secretion during induction with 25 to 40 per cent cyclopropane in the dog. Ether and chloroform produced greater secretory activity, nitrous oxide and ethylene none. Salivation was entirely suppressed during deep anesthesia with all agents by their action on the secretory center. Little is known concerning the effect of cyclopropane, or for that matter any other agent, on the secretion of mucus. It is the latter, rather than the serous secretions, which are troublesome in practical anesthesia.

Cyclopropane is the only one of the commonly used agents, either gaseous or volatile, which does not produce an increase in respiration, even during induction (106, 117). Usually a slight and progressive decrease in rate, but particularly in tidal volume, occurs, until intercostal paralysis is complete. At this time, a small increase in the concentration of cyclopropane will effect respiratory arrest. The increase in rate which usually occurs with other agents, just prior to respiratory arrest, is slight, or lacking, with cyclopropane, thereby making it difficult to differentiate third and fourth plane surgical anesthesia. The decrease in minute volume respiration is accompanied by an increase in the carbon dioxide content of arterial blood (117) and tissues (105). If the concentration is increased gradually, respiration invariably fails before the circulation, and recovery occurs readily if artificial respiration is instituted, and the concentration diminished.

Cyclopropane in anesthetic concentration relaxes the peripheral blood vessels. The evidence for this statement is the fact that venous blood becomes arterialized, even when the inspired oxygen is 21 per

cent (117). Capillary bleeding also appears to be increased. It is not uncommon for blood pressure to rise during anesthesia with this gas. This rise is probably not due to a direct action of cyclopropane since it may usually be explained on the basis of an accompanying retention of carbon dioxide, anoxia, or pressor reflexes which arise from sensory stimulation under light anesthesia. If care is taken to eliminate these factors, a rise is seldom seen. This phase of its action needs further study. The heart rate is usually reduced below the resting level during anesthesia in man. It has been stated that the same is true for the dog (106), but wider experience indicates that such a conclusion is not justified although a slowing occurs in some animals.

In the early experimental work with this gas, Lucas and Henderson and Waters both noted that high concentrations of cyclopropane produced cardiac arhythmia in animals and man. Seevers et al. (106) observed that the irregularities occurred just prior to, or following, respiratory arrest in the dog, at concentrations of cyclopropane between 35 and 40 per cent. Under artificial respiration, a higher concentration of cyclopropane (average 47 per cent) was required to initiate arhythmia. Robbins and Baxter (98) have confirmed this observation. The irregularities occurring prior to respiratory arrest are A-V blocks and extrasystoles at relatively slow rates. Seevers et al. thought the early arhythmia to be of vagal origin (escape phenomenon) since it was prevented by atropine. Anoxia was found to contribute to the early appearance and persistence of arhythmia. Robbins and Baxter (98) concluded that the cardiac irregularities which develop at the time of, or soon after, respiratory arrest in the dog are not due to cyclopropane but to anoxemia, since, with but one exception, no arhythmia was observed in their series of 13 dogs until some minutes after respiratory arrest. When irregularities did occur, the average oxygen content of arterial blood was only 2.8 per cent. They also observed that vagal section did not prevent the arhythmia which occurred after respiratory arrest, but prevented the increase in the P-R interval, and the more severe types of A-V block.

The reviewers are not willing to accept anoxia as an explanation of all the arhythmia which occurs during cyclopropane anesthesia, even though it has been shown to be a factor, and undoubtedly plays a major rôle in very deep anesthesia accompanied by respiratory depression. At present the information concerning the action of this compound on the vascular system, the coronary vessels, and the myocardium is too meager, and recent observations do not lend support to such a conclusion. The "multiple focus ventricular tachycardia," seen by Kurtz,

Bennett and Shapiro (60) in four of forty-one clinical administrations of cyclopropane which were purposely carried to fourth plane anesthesia, and the high incidence of irregularities of ventricular origin in the dog experiments (98, 106), seem to be indicative of an increased irritability of the automatic tissue of the heart. Meek, Hathaway, and Orth (72) have recently found the stimulating, or sensitizing, effect of cyclopropane on the automatic tissue of the dog's heart to exceed that of ether or chloroform, as judged by the appearance of ventricular tachycardia after a standard dose of epinephrine. They found the irritability of the automatic tissue to be directly related to the concentration of the anesthetic agent, and state:

> In the adrenalin controls A-V blocks were common and the ventricular rhythms were slow escape phenomena in nature. When the heart was under the influence of cyclopropane, however, the ventricular specialized tissue was so heightened in its irritability that when adrenalin reached it an automatic rate appeared that was dominant over the sinus. The ventricular tachycardias were almost always at a rate of 300 per minute.

The significance of these electrocardiographic findings is difficult to evaluate from a practical standpoint. Irregularities are a common clinical occurrence with all types of anesthesia including procaine blocks. In the series of Kurtz et al. (109 cases with all agents), only 21 per cent failed to show some disturbance of rhythm. The frequency of ventricular tachycardia with cyclopropane is noteworthy, since only a slight increase in irritability may result in ventricular fibrillation. The use of epinephrine, and presumably all sympathomimetic or other drugs which increase the irritability of the automatic tissue, is apparently contraindicated in cyclopropane anesthesia. The record of a clinical case has been made available to the reviewers in which the accidental intravenous injection of 0.4 mgm. of epinephrine produced cardiac death during cyclopropane anesthesia. This dosage is of the same order as those used by Meek et al. Do these electrocardiographic changes indicate myocardial damage, and if so is it transient or permanent? As yet, no functional or pathological studies have furnished the answer to this question. The experimental and clinical evidence would point to the transient nature of these changes. In the hands of those familiar with its use, cyclopropane anesthesia is accompanied by fewer unfavorable postoperative reactions than with other agents of similar potency, even in bad risk and cardiac cases.

Peoples and Phatak (87) report an increased tone of isolated rabbit jejunum when exposed to 10 to 25 per cent cyclopropane in oxygen.

Meek (117) has found that both the circular contractions and propulsive phenomena are inhibited or arrested in normal dogs with Thiry or Thiry-Vella fistulae during deep cyclopropane anesthesia. Prompt return to normal or increased activity occurred when anesthesia was terminated. Seevers (103) observed that the ten minute inhalation of 6 per cent cyclopropane inhibited hunger contractions in man without affecting gastric tone. These contractions returned in full strength when inhalation ceased and progressed to the usual tetanic contracture.

A summary of 10,600 administrations (117) recently published, indicated the incidence of nausea or vomiting to be: nitrous oxide, 23 per cent; ethylene, 35 per cent; cyclopropane, 39 per cent; ether, 41 per cent. In this same group the incidence of postoperative distention was: nitrous oxide, 3 per cent of 1,300 cases; ethylene, 7 per cent of 1,000 cases; cyclopropane, 13 per cent of 5,800 cases; ether, 16 per cent of 2,400 cases. In this series, nitrous oxide or ethylene were seldom used for abdominal operations, or the incidence with these agents would have been higher.

The action of cyclopropane on the uterus has not been studied experimentally. The progress of labor can be arrested by deep anesthesia, while the rapid recovery of tone as anesthesia is lightened tends to diminish postpartum hemorrhage (117).

Henderson and Lucas (41) noted no significant change in either the pH or carbon dioxide combining power, and but little rise in blood sugar of cats and rabbits during cyclopropane anesthesia. The non-protein nitrogen and the carbon dioxide combining power of blood were not significantly affected in 21 clinical administrations, although a ten per cent rise in blood sugar and a seventy-five per cent elevation in blood phosphorus occurred. Neff and Stiles (77) observed an 8 to 30 per cent rise in the blood sugar of 25 patients, no change in non-protein nitrogen, and a constant lowering of the carbon dioxide combining power by 2 to 7 volumes per cent. These same workers found an average blood sugar rise of only 7 mgm. per cent, and no change in carbon dioxide combining power, of 30 controlled diabetic patients anesthetized with cyclopropane. Urinary output is usually suppressed during cyclopropane anesthesia and a compensatory increase in excretion occurs several hours following anesthesia. In this respect, the effect is similar to that caused by ether and ethylene and not more marked (118). Further study is needed concerning the action of cyclopropane on water balance.

Raginsky and Bourne (88) found no liver injury in dogs, after repeated daily administration (1 hour) of cyclopropane for 9 days, as judged by

the capacity to eliminate bromsulphalein, and by histological examination. Neither did cyclopropane have an adverse effect upon the rate of recovery from hepatic injury produced by chloroform, as judged by the same criteria. These authors could find no evidence of additional hepatic injury to eclamptic patients who gave evidence of such injury by dye retention.

Taylor and Waters (112) found that cyclopropane, like other agents (nitrous oxide, ethylene, ether) produced an absolute and relative increase in the leucocytes of peripheral blood which persisted for several days after anesthesia in man. That this rise was not due to trauma is indicated by the fact that unoperated dogs show similar changes. Only very slight changes were observed during operations under spinal anesthesia. No consistent changes in red cell volume or coagulation time occurred.

Although no clear-cut histological evidence of parenchymatous injury by cyclopropane has been presented, the question is not settled, particularly in view of the functional evidence of alteration in cardiac automaticity. Such a study is desirable, but in order to be convincing, the evidence must be obtained on animals living under carefully controlled dietary conditions.

Methyl cyclopropane. This compound has recently been studied by Henderson and McDonald (42), along with the 1:2 dimethyl, and the trimethyl derivatives of cyclopropane. The latter compounds are liquids at ordinary temperatures. Methyl cyclopropane in concentrations of 14 to 15 per cent produces deep anesthesia in the cat. The appearance of clonic movements, and cardiovascular depression, as judged by the fall in blood pressure and the appearance of arhythmia, apparently condemn it as an anesthetic of value. It is interesting to note that the anesthetic potency and toxicity of these derivatives, like the straight chain and the unsaturated hydrocarbons, increases with the addition of each carbon atom, the order of potency as indicated by average effective anesthetic concentrations being roughly, cyclopropane 20; methyl cyclopropane 15; dimethyl cyclopropane 10; and trimethyl cyclopropane 7 or less.

The diolefines (C_nH_{2n-2})

	MOL. WT.	B. P.	SP. GR.	ANESTHETIC RANGE
		°C.	*air = 1*	*vols. per cent*
Allene—CH_2:C:CH_2	40	−22	1.39	20–35
1:3 Butadiene—CH_2:CHCH:CH_2	54	−5	1.87	10–15

The following data concerning the anesthetic properties of these compounds is taken from the papers of Schmidt and Shaumann (102) and Killian (54).

Allene (propadiene) A colorless gas, with an acetylene-like odor and a sour after-taste, which tends to change chemically on storage. Absorption coefficients, at 20°C. are: water 0.304; blood 0.440; and sesame oil 8.271 (54). Oil-water coefficient of 27. Studies on the mouse indicate incomplete narcosis with 30 per cent, death at 40 per cent. Reflex activity is abolished in the rabbit with 50 per cent allene, the blood pressure is elevated, the pulse rate increased, and respiration is stimulated both in rate and depth. No changes were noted in the electrocardiogram. It is interesting to note that the solubility coefficients of this gas are very similar to those of cyclopropane, and that the anesthetic range is likewise of the same order.

1:3 Butadiene. The odor of this gas is so disagreeable as to render it unfit for anesthesia in man. It is of interest, however, from the standpoint of comparative pharmacology. Absorption coefficients at 20°C. are: water 0.350; blood 0.870; sesame oil 37.32. Oil-water coefficient of 107. Anesthesia in the mouse is accompanied by an increase in reflex excitability. Excessive salivation, hyperventilation, a marked increase in pulse rate, and a disturbance in cardiac rhythm, characterize anesthesia in the rabbit.

The chemical instability of this group of compounds, their known tendency to polymerize on standing, their unpleasant odor, the small margin of safety, the heightened reflex excitability, and evidence of cardiac injury, would appear to render them unsatisfactory as anesthetics for man.

The acetylenes

	MOL. WT.	B. P.	SP. GR.	ANESTHETIC RANGE
		°C.	*air = 1*	*vols. per cent*
Acetylene—CH:CH	26	−88.5	0.92	50–85
Allylene—$CH_3C{:}CH$	40	−23	1.39	

The presence of the triple bond renders these compounds highly reactive chemically and limits in some measure their practical use. No references to the pharmacological study of allylene have been found Should this gas be proven to be a good anesthetic agent for laboratory animals, the interest would be largely academic, since it possesses a

vile odor. Certain other gaseous acetylenes (ethyl acetylene, diacetylene, and vinyl acetylene) are apparently too unstable to merit consideration. Acetylene has been used quite extensively in central Europe and the majority of published material, both experimental and clinical, appears in the German literature. Kochman (59) has recently reviewed the pharmacological literature pertaining to this agent and Killian (53) discusses its clinical use.

Acetylene. A colorless gas with an unpleasant, pungent, ether-like odor and a sweet, or bitter, after-taste. The gas has a high range of explosiveness: in air, 2.8 to 40 per cent; in oxygen 2.8 to 85 per cent. Spontaneous explosion may occur at two atmospheres' pressure. It is stored in solution in acetone, and the commercial gas contains impurities (phosphides, arsine, etc.) which must be removed before use as an anesthetic. The pure gas is marketed under the name of Narcylene and must be passed through water, or over activated carbon, to remove the acetone vapor. The latter is a distinct hazard unless removed and care must be taken in this regard.

The absorption coefficients at 20°C. are: water 1.042; blood 0.965; sesame oil 2.324 (54). The oil-water coefficient is 2.2. It will be noted that the blood solubility is less than the water solubility. These values were obtained by Killian for defibrinated ox blood, and the same ratio obtains for rabbit and human, but not for dog's blood. Grollman (34) has shown that these differences may be explained by the variations in the water and lipoid content of different bloods.

Acetylene is absorbed and eliminated by the body quite rapidly. Schoen (100) obtained complete body saturation of the rabbit in 17 minutes, as judged by blood analysis for acetylene. Similar values were obtained for desaturation by Schoen and Slikwa (101), who found that the blood was acetylene free in twenty minutes, 86 per cent of the gas having been eliminated within the first minute. The diffusion rate is undoubtedly rapid as both Führer (27) and Teschendorf (113) have shown that 100 cc. of acetylene disappears from the peritoneal cavity of the rabbit within thirty minutes.

The early attempts to determine effective narcotic concentrations yielded erroneous results because of the presence of impurities in the gas used. A survey of the literature might indicate a considerable species variation in response to the narcotic action of this agent. This is probably due to the use of different criteria of anesthesia. Concentrations of 50 to 80 per cent will induce varying degrees of narcosis in mice (54), rats (93), guinea pigs (120), rabbits (54), cats and dogs (28,

46, 47, 62). It is necessary to use a concentration of 70 to 80 per cent for surgical anesthesia in man (53).

Davidson (16) studied the effects of low concentrations on the response to simple psychological tests. She noted that consciousness was retained if the concentration did not exceed 30 per cent. At 35 per cent, unconsciousness occurred in five minutes. Killian (53) finds the following concentrations effective in clinical anesthesia: analgesia 30 to 40 per cent; excitation 50 to 60 per cent; third stage-plane one 60 to 70 per cent; plane two 70 to 80 per cent. In the latter plane, some degree of abdominal relaxation may be obtained. It is impossible to reach a lower plane without the simultaneous occurrence of anoxia. There is no third plane of anesthesia with this gas, according to the usual classification of signs (36), since it is not capable of producing intercostal muscle paralysis. Concentrations above 85 per cent produce an increase in respiratory activity and muscle rigidity returns. This gas, like nitrous oxide and ethylene, will not furnish satisfactory relaxation in the majority of instances.

Most investigators agree that acetylene is too weak a narcotic to paralyze respiration without the aid of oxygen want. All of the signs of anesthesia which occur with concentrations above 85 per cent are complicated by the presence of oxygen want. Respiration is usually increased throughout anesthesia (53), the amplitude being affected more than the rate. A steady increase in ventilation occurs as the concentration is increased, until it reaches a stage which Killian terms "toxic hyperventilation." According to Rehn and Killian (90), the sensitivity of the respiratory center to carbon dioxide is not altered by this gas. The carbon dioxide tension in blood is usually lowered (28, 101).

A rise in blood pressure usually occurs in full narcosis. In fact, Killian (55) and others (26) state that the action of this agent tends to combat shock!! This rise in blood pressure is due to the simultaneous increase in the cardiac output and a central stimulation of the vasomotor center. Both the resulting splanchnic constriction, and the increase in blood flow through the peripheral capillaries, have been demonstrated by plethysmographic studies. The cardiac slowing which occurs in surgical anesthesia is of reflex origin, and occurs secondarily to the increase in blood pressure (26, 47).

The alkali reserve in the rabbit (28), dog (101), and man (114) is lowered. Fuss and Derra (28) found an increase in the lactic acid content of blood in some cases. Blood sugar is elevated, probably in relation to the increase in fixed acids. No significant changes occur in the

formed elements of the blood (76) and prolonged exposure, even to 93 hours of anesthesia, yields no evidence of parenchymatous damage (25).

The ethers

	MOL. WT.	B. P.	SP. GR.	ANESTHETIC RANGE
		°C.	*air = 1*	*vols. per cent*
Methyl ether—$(CH_3)_2O$	46	−23.6	1.61	60–85
Methyl ethyl ether—$CH_3 \cdot O \cdot C_2H_5$	60	+7.9	2.10	

Dimethyl ether was introduced by Richardson as an anesthetic in 1867 (69). He considered it a good anesthetic. He states that rabbits and pigeons remain in a full anesthetic mixture for 12 minutes without dying and after being allowed to "die" may be resuscitated by artificial respiration seven minutes after cessation of life. He used it in 27 human cases. No mention can be found of the use of methyl ethyl ether although Richardson dissolved methyl ether in ordinary diethyl ether and used the mixture as an anesthetic.

Dimethyl ether. A colorless, inflammable gas, with an unpleasant odor somewhat resembling chloroform. It is difficult to inhale and produces a sense of oppression or suffocation. It is quite soluble in water, one volume absorbs 37 volumes of the gas (69). Brown (6) studied its action on the cat. He found that a concentration of 65 per cent was necessary to induce satisfactory anesthesia while profound anesthesia with gradual respiratory arrest occurred with eighty-five per cent. Recovery time was long, twenty minutes being required for complete recovery after fifty minutes of anesthesia. Similar results were obtained by the authors (119) on the dog. Excessive salivation occurred, and it was impossible to induce deep anesthesia on this animal without the presence of running movements, and other evidence of muscular activity. Davidson (19) studied the effects of the gas on normal subjects. The inhalation of 20 per cent methyl ether produced unconsciousness in seventeen minutes. These experiments would also indicate a slow absorption and elimination of the gas. With concentrations above 10 per cent, the depression persisted for a considerable period. Nausea occurred at this concentration, but no vomiting. Brown (6) inhaled concentrations up to 50 per cent but does not describe the effects. The reviewers do not believe this agent to be worthy of extended study with a view to its practical use.

DISCUSSION. The narcotic gases as a class possess a greater lability of action than any other group of anesthetic compounds. The short induction and recovery time, and the rapidity with which changes in the degree of anesthesia may be effected during maintenance, are of great advantage to the practical anesthetist. The rapid diffusion of the gases is made possible by their small molecular size, the low boiling point, their characteristic solubilities and the fact that they pass through the body unchanged. It is unlikely that a volatile or non-volatile agent will be found which can compete with the gases for rapidity or lability of action. Compounds which are dependent upon a rapid destruction in the body, or renal elimination, for their brevity and lability of action, do not appeal to the reviewers as being possessed with the necessary requirements of safety to be of universal value as general anesthetic agents. This latter class of compounds (barbiturates, tribromethanol, etc.) are generally considered to serve best as agents for premedication. If so used, their depressant action may be advantageously supplemented by the narcotic gases, without sacrificing completely the control of the state of reflex excitability of the patient. Certain generalizations, many of which have been pointed out by Killian (54) may be made regarding the hydrocarbon series of gases. Some of these statements apply also to nitrous oxide.

The narcotic strength and toxicity increases in each of the homologous series with the addition of each carbon atom to the molecule. With this increase in strength, there also occurs a diminution in the margin of safety.

An increased solubility in oil parallels the increase in the number of carbon atoms and narcotic strength. Although in most instances the solubility in water and blood increases in each homologous series, there are too many exceptions to permit generalization.

An increase in the excitability of nervous and automatic tissue occurs with the addition of each carbon atom in every homologous series. This is manifested by an increasing muscle rigidity and spasm, hyperventilation, and cardiac arhythmia, all of which occur during deep narcosis.

The presence of double bonds in the molecule increases the narcotic strength. The narcotic potency becomes progressively greater with propane, propylene and propadiene (allene); and also with butane, butylene, and butadiene. It may be that this increase in action is due to the parallel increase in lipoid solubility.

A triple bond increases slightly the narcotic strength. Compare, for example, ethylene and acetylene.

Branching of the carbon chain decreases narcotic strength as is evidenced by the fact that isobutylene and isobutane are approximately one-half as effective as normal butylene and normal butane. In this respect, the gases are similar to the alcohols but differ with most alkyl derivatives of barbituric acid. In the case of isobutylene, the oil and water solubilities are only half those of normal butylene.

To be a satisfactory anesthetic, a gas must be one with a "sufficient solubility in water to be carried to the central nervous system and such a high degree of solubility in lipoids as to lead to its differential accumulation there." (10) With the exception of acetylene all of the hydrocarbons have a greater solubility in blood than in water. All of the gases discussed here except the lower members of the methane series have an adequate solubility in water to reach the nerve cells.

Obviously, it would be absurd to attempt to use the oil-water coefficients as indices of brain-blood distribution coefficients, since there is little reason to believe that the lipoids of nerve tissue have affinities for the gases which are identical with their oil solubilities. Further, the lipoids only represent a relatively small fraction of the total constituents of the nerve cell. The oil-water coefficient does give, however, a rough index of the diffusion rate of the different gases. This statement does not imply that induction or recovery time can be correlated with the rate of diffusion of a gas, since the absolute solubility in water and lipoids controls the total quantity of the agent in solution in the body. With gases of high lipoid solubility, saturation and desaturation are delayed. There is no doubt but that a good correlation can be made between narcotic potency and oil solubility with the gases, as with the volatile agents. This parallels, if it does not explain, the ineffectiveness of the lower members of the methane series.

No evidence has been presented to indicate that the gases discussed here are modified chemically in their passage through the body. It is questionable whether a narcotic gas would be of practical value, if it were chemically unstable during storage or underwent change in the body during anesthesia.

With the possible exception of nitrous oxide, none of the narcotic gases form combinations with hemoglobin which may be detected spectroscopically.

The higher members of the olefine series and the diolefines are difficult to purify. It is probable that the presence of small quantities of high boiling point fractions in these gases has modified the toxicity values which have been presented for these compounds. The same possibility

exists regarding the changes which occur with agents like cyclopropane. The removal of the last traces of these contaminants is a difficult chemical and manufacturing problem, and it is always possible, particularly with unsaturated compounds, or with saturated compounds which resemble them chemically, that their polymerization may be effected by the catalytic action of the metal of their containers.

All of the hydrocarbons are inflammable and explosive. From a practical standpoint, the explosive range is of relatively little importance as a guide to safety, since some time during anesthesia it must be expected that an explosive mixture will be present either in the breathing bag, mask, the gas machine, or their vicinity.

The number of existing gases is quite small, most of the promising ones have been studied for their narcotic properties, and the diminutive size of the gas molecule apparently limits the number of possible atomic combinations. In spite of these facts, which might be regarded with pessimism by potential investigators in the field, the reviewers retain hope that future search may yield a new gaseous narcotic which is superior to those in common use. In their opinion, the ideal anesthetic agent should be a gas.

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